

University of Bath



PHD

Cadmium biosorption by bacteria

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Award date:
1988

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Cadmium Biosorption by bacteria.

submitted by

Stephen Palmer

for the degree of Ph.D of the University of Bath

1988

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SUMMARY

The accumulation of cadmium by seven bacterial strains was studied to assess their suitability for removal of cadmium from waste-water. Cadmium uptake was very rapid and fitted a Langmuir adsorption isotherm. Temperature, pH, presence of other metal ions and biomass concentration all affected cadmium biosorption. Viable biomass was employed as standard but investigation revealed no major difference in cadmium biosorption between live and dead biomass. T.E.M and X-ray spectroscopy showed that sites and mechanisms of cadmium uptake differed between bacteria. Production of extra-cellular polysaccharide enhanced cell survival and effected cadmium uptake. Bacterial exopolysaccharide was a good biosorbent. Cadmium recovery from biomass was achieved with several chemical eluants. Desorption of cadmium was as rapid as uptake. Cadmium removal from solution within fluidised beds was achieved with both A.viscosus and K.aerogenes 9128.

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Acknowledgements

I would like to thank my parents for their support throughout my education, Dr Ashley Scott for guidance during this course of study, Mr Dave Powell and Miss Gill Sage for technical assistance and Dr J.Berriman for electron microscopy. In addition I would like to thank Bath University for providing my grant, Prof. J.Howell for use of departmental facilities and Philippa for typing this thesis.

1. Introduction.

1.1 Introduction

In recent years a growing interest has developed in the use of microorganisms in recovery of metals. Over the past twenty years or so since the natural role of some bacteria in metal leaching has been exposed, bacteria are now purposely being used for extraction of metals from low grade ore (Brierly, 1982; Manchee, 1979). The minerals industry has also always been faced with the problem of dealing with metal-contaminated waters. This has no doubt contributed to the metal mining industry being among the first to apply the process of microbial metal accumulation to wastewater treatment on a large scale. In New Mexico the Kerr McGee Corporation successfully reduced uranium levels in their mine discharge waters by having them flow through a series of algae-containing ponds (Brierly and Brierly, 1980). In Missouri, Gale and Wixson (1979) reported the use of tailings ponds and meander systems containing algae which successfully reduced lead in the mining and milling effluents for seven years.

Thus the recovery of toxic heavy metals by microorganisms has been practised for some time. At present the main use for microbiological removal of metals from wastewater is decontamination. Despite the general rise in costs of obtaining most metals, in most cases they are not so scarce as to make recovery of the metal for its own sake a commercial proposition. One

noteable exception to this generalisation does, however, exist. Both electrolysis and ion exchange have been employed on an economically viable basis for recovery of silver (Thompson and Prior, 1984)

1.2 Microbiological heavy metal recovery - main areas of application

At present the major area for application of heavy metal extraction by microbial biomass is wastewater treatment. Many heavy metals are toxic, giving rise to environmental concern over their release into the biosphere. These metals, which include zinc, lead, nickel, chromium, copper and cadmium, cause problems for U.K. Water Authorities as their levels in effluent are required to be finely controlled. In addition, these metals are accumulated by the microorganisms that constitute the sewage sludge of sewage treatment plants. Such accumulation can cause severe problems with disposal of sewage sludge through the traditional route of spreading on agricultural pastureland. Presence of toxic metals within sludge leading to crop uptake has precluded this cheap means of disposal for some water authorities (Thompson and Prior, 1984). Cadmium in particular is highly toxic and has caused problems in sludge disposal (Dart, 1982). In extreme cases, the toxic effects exerted by certain heavy metals may also change the microbiota of activated sludge plants and

inhibit sewage treatment resulting in poorer quality effluents (Brown and Lester, 1979). For U.K. Water Authorities the most practicable control of toxic metals would be achieved by pretreatment of wastewater at the factory of origin (Foster, 1983).

A further field of application of biological metal recovery is the treatment of nuclear waste ('Biotechnology, 1983; Tsezos, 1981; Ruchloft, 1949). Throughout the period that metal uptake by microorganisms has been investigated, radionuclide and uranium recovery in particular, has been a major area of interest (Brierly, 1986).

Of the non-radioactive toxic metals known to pose environmental hazards, one of the most dangerous is cadmium (Lauwerys, 1979; Samarawickrama, 1979; Webb, 1979; Peterson and Alloway, 1979). Whereas much work has been carried out on uranium, comparatively little to date on microbial uptake of cadmium has been completed.

This particular course of study grew from a recognition of this, and therefore concentrated specifically on cadmium.

1.3 Interactions between metals and microorganisms

In order to understand cadmium accumulation in

microorganisms, the interactions between metals and microorganisms in general need to be considered.

1.3.1 The requirement for some metals by microorganisms

Very low concentrations of certain metals are required by all microorganisms for normal cellular functioning. These include potassium, magnesium, manganese, calcium, iron, cobalt, copper, zinc and molybdenum. For example, copper, zinc and molybdenum are constituents of specialised enzymes. Cobalt is found in vitamin B₁₂ and its coenzymes. Magnesium, iron, manganese, calcium and potassium are also enzyme cofactors (Stanier et al, 1979).

Of these metals, copper, iron, potassium and magnesium are required to a greater degree than the others which are usually required only in trace amounts. These micro-nutrients are often in fact toxic at high concentration (Sterritt and Lester, 1980). Cadmium, however, has no known metabolic role.

1.3.2 Metal toxicity and microorganisms

High levels of heavy metals in the environment are usually toxic to microorganisms. Some microorganisms may even be affected by quite low concentrations of particularly toxic metals. For the overall cell population toxicity may manifest itself as a drop in cell numbers due to cell death, bacterostasis, or

extension of the lag phase of the cell cycle (Sterritt and Lester, 1979). If bacteriostasis, or a lengthened lag phase occurs, cell metabolism is interfered with, but not severely enough to cause cell death (Tuovinen and Kelly, 1979). Heavy metal toxicity may also manifest itself in altered cell morphology (Ehrlich, 1978). The toxic metal is likely to interfere with transport systems within the cell (Maxwell et al, 1971). This may be a result of interference with cell function by protein denaturation (Gadd and Griffiths, 1978), disruption of enzyme structure and disruption of DNA (Sterritt and Lester, 1980).

In general the toxicity of a heavy metal is determined by its degree of attraction to natural metal binding sites on and within the cell. The similarity in chemistry of some heavy metals to other elements required for cellular functioning may result in some being actively accumulated within the cell. In general, the ability of a toxic metal to penetrate through to the cell cytoplasm is a significant measure of its potential toxicity (Sterritt and Lester, 1980).

However, metal toxicity is mediated by several factors. The nutritional state of the organism may alter toxicity as cells in a nutrient-depleted environment are often more susceptible to metal toxicity. Environmental

factors heavily influence heavy metal toxicity and some of these are reviewed below.

1.3.3 Influence of the environment on metal toxicity

The presence of metal chelating compounds, other ions, and pH of the environment all affect the toxicity of heavy metals to microorganisms.

Other cations, particularly those of similar ionic radii, can decrease toxicity due to competition for binding sites, (Gadd and Griffiths, 1978; Sterritt and Lester, 1980; Silver and Misra, 1983; Tuovinen and Kelly, 1974 a. and b.).

Low pH, i.e. high hydrogen ion concentration reduces metal toxicity (Gadd and Griffiths, 1978; Sterritt and Lester, 1980), probably due to ionic competition between hydrogen ions and metal ions, (Friis and Myers-Keith, 1986). High pH may enhance metal toxicity (Babich and Stotzky, 1977), due to low hydrogen ion concentration leading to less ionic competition. But for some metals, increase in pH beyond a particular point may lower toxicity because of precipitation removing metal from solution, (Tuovinen and Kelly, 1974 a., 1974 b.).

Agents capable of chelation can affect toxicity by binding the metal. For example, in nature Kaolinite and

montarillonite clays can reduce heavy metal toxicity by binding the metal. Humic, fulvic acids and proteins can also have the same effect (Gadd and Griffiths, 1978). The presence of synthetic chelating agents such as E.D.T.A. have been shown to reduce heavy metal toxicity toward microorganisms (Sterritt and Lester, 1980).

1.3.4 Resistance to metal toxicity by microorganisms

Microorganisms exposed to adverse environmental conditions may soon produce strains capable of surviving in a hostile environment through genetic modification. In many cases the evolved mechanisms are highly specific (Silver and Misra, 1983). In bacteria this metal resistance is often plasmid-linked (Hardy, 1983; Iverson and Brinckman, 1978; Ehrlich, 1978) and often associated with antibiotic resistance (Hardy 1983; Allen et al, 1977; Nakahara et al, 1977).

Two general strategies exist for achieving resistance to toxic metals:

- (i) increase impermeability of the cell to the metal
- (ii) biochemically achieved transformation of the metal.

The former process protects the cell from toxic elements in its environment. The latter detoxifies the immediate environment of the cell by eliminating the toxic metal from it or altering it to a non-toxic form, (Silver and

Misra, 1983; Ehrlich, 1978; Iverson and Brinckman, 1978).

Increased impermeability may be achieved non-specifically by production of an outer protective layer around the cell. This allows some metal to be bound at a distance from the cell wall with little damage being caused, (Corpe, 1975). This non-specific mechanism appears to be employed by the bacterium Zooglea ramigera, a common member of sewage sludge microbiota. Comparison of metal toxicity on strains of Z. ramigera capable of producing extracellular polysaccharide around the cell with that of a strain incapable of exopolysaccharide indicated that the former fared better in metal contaminated solutions and also accumulated more metal than the latter (Friedmann and Dugan, 1968). Encapsulated strains of Azobacter have been found to survive better in lead rich solutions than non-capsule producing Micrococcus luteus due to the former's ability to immobilise lead without the metal being able to exert toxic effects at the cell surface or intracellularly. Some periphytic pseudomonads have been found to take up copper predominantly in their extracellular polymer, with only a fractional amount actually reaching the cell.

Capsulate strains of Klebsiella aerogenes were

found to survive in 10mg/l cadmium better than a strain that did not secrete extracellular polysaccharide around the cell. Furthermore, when capsular polysaccharide was separated from polysaccharide producing strains and added to non-producing strains in cadmium solution, the survival of the latter was enhanced (Bitton and Freihofer, 1978).

A layer or matrix of extracellular polymer therefore appears to enhance cell tolerance of toxic metals by immobilising them away from the immediate proximity of the cell where they cannot bind to functional groups on the cell surface or within the cell. It should be noted, however, that extracellular polymer capsules and matrices may not have evolved specifically to protect bacterial cells from toxic metals: they are also known to offer resistance to phagocytosis ingestion by amoebae or phagocytes, protect against bacteriophage and dessication and might also act as a food reserve, (Wilkinson, 1957).

More specific resistance mechanisms to toxic metals are known in which cellular permeability to the metal is decreased. Some strains of Staphylococcus aureus are more resistant to cadmium toxicity than others, due to an alteration of the specific transport system responsible for bringing cadmium into the cell. Some

Eschericia coli strains are cobalt resistant due to a change in the specific uptake system responsible for translocation of cobalt (Sterritt and Lester, 1980).

The alternative strategy to increasing cell impermeability is transformation of a toxic metal into a non-toxic form. This may be achieved intracellularly, but is more commonly achieved extracellularly. Alternatively, a toxic metal may be transformed into a form that is inassimilable by the microorganism. Toxic metals may be oxidised, reduced or methylated to produce less toxic compounds. Mercury resistance is often plasmid-linked via a plasmid determined enzyme which can transform mercury and organo-mercurials into volatile forms which are soon lost from the environment, (Silver and Misra, 1983). Another mechanism for removing metals from solution is production of hydrogen sulphide by microorganisms. As most heavy metals form insoluble sulphides, the production of sulphide by the bacterium Desulphovibrio desulphuricans, the fungus Poria vaillantii and some strains of the yeast Saccharomyces cerevisiae results in precipitation of the metal from solution (Gadd and Griffiths, 1978). Some fungi are also capable of producing chelating agents which bind metal away from the cell. Corrollus palustris, among others, can produce oxalic acid to enhance its copper

tolerance by this means (Gadd and Griffiths, 1978).

Thus it can be seen that many mechanisms exist by which microorganisms may enhance their tolerance of toxic

metals. One significant point that should be borne in mind when considering application of a metal tolerant microorganism to toxic metal recovery is that some of the mechanisms of resistance are not compatible with recovery of the metal.

1.4 Accumulation of heavy metals by microorganisms

Several mechanisms exist by which microorganisms remove heavy metals from solution. These may be divided into two general categories: metabolism dependent uptake into the cell and binding of metal ions to extracellular material (e.g. capsular polymer), or the cell wall which is not an active process (Shumate and Strandberg, 1985; Gadd, 1986; Kelly et al, 1979). Some potentially toxic metal ions have already been previously mentioned to be micronutrients at low concentration. Most are divalent metal ions (for example, Alcaligenes eutrophus exhibits a growth requirement for nickel) and active uptake systems exist to bind these ions.

These divalent cation uptake systems tend to be particularly specific : however some do transport metals into the cell apart from those primarily required. The magnesium uptake system of E.coli is suspected also to accumulate Ni^{2+} , Co^{2+} and Zn^{2+} . The Mg^{2+} transport system of Saccharomyces cerevisiae is known to take up Co^{2+} , Mn^{2+} , Zn^{2+} and Ni^{2+} . Generally, ion uptake systems are specific for ions of a certain ionic radius. Thus monovalent cation uptake mechanisms tend not to take up divalent metal ions or metal ions of a higher valency, excluding the toxic heavy metals. However, caesium and radio isotopes of caesium and Tl^{+} have been observed to be taken into the cell via the potassium transport system (Kelly et

al), 1979).

Anion transport systems have also been implicated in carriage of toxic metals into cells. Metals that exist as oxanions in solution may be accumulated by such systems. Chromate for example, has been demonstrated to be competitive with sulphate ions for uptake via the sulphate permease system of Neurospora crassa. Many of the cases of intracellular uptake of toxic metals known are active processes, but intracellular uptake of toxic metal by non-viable cells is also known to occur (Strandberg et al, 1981).

The term 'biosorption' has been coined to describe the non-active adsorption of heavy metal ions by microorganisms or biological polymers. This process has been defined by Shumate and Strandberg (1985) as "the non-directed, physical-chemical complexation reaction between dissolved metal species and charged cellular components, akin in many respects to ion exchange . Such processes usually occur as interactions between negatively charged ligands and metal ions and may occur as ion-exchange or formation of complexes. The most likely components of microbial polymers capable of ion exchange are carboxyl groups, organic phosphate groups and organic sulphate groups. Chelation or complex formation tends to occur on biopolymers where neutral

divalent oxygen, sulphur atoms or trivalent nitrogen atoms are present. Examples include amino- and imino- and heterocyclic nitrogen groups of proteins and nucleic acids and also the carbonyl and hydroxyl oxygens of the same polymers. The latter two groups are also found in polysaccharides, polyheterocyclics and polyphenolics (Hunt, 1986). As previously mentioned, extracellular polymers have been demonstrated to bind heavy metals, such as the binding of metal to the extracellular polymers produced by the bacteria Z. ramigera and K. aerogenes. Extracellular accumulation of metals has also been demonstrated to occur with the extracellular polysaccharides of the algae Mesotaenium kramstei and Mesotaenium caldariorum (Mangi and Schumacher, 1979).

Accumulation of metal at or within the cell surface has been observed to occur with many microorganisms. The bacteria Bacillus subtilis (Beveridge and Murray, 1976), Bacillus licheniformis (Beveridge et al, 1982) and Escherichia coli (Beveridge and Koval, 1981) have been demonstrated to bind heavy metal ions to their cell surfaces. Among the fungi Saccharomyces cerevisiae (Strandberg et al, 1981), Neocosmospora vasinfecta (Gadd and Griffiths, 1978) Rhizopus arrhizius (Tsezos and Volesky, 1981) Neurospora crassa and a Penicillium species (Gadd, 1986) have all bound metal to their cell walls. Considerable diversity exists between the cell wall

composition of bacteria and fungi, yet all apparently contain groups capable of metal binding.

Matthews et al (1979), Beveridge and Murray (1980) and Doyle et al (1980) have identified the predominant divalent metal ion binding group in Bacillus subtilis cell walls as the glucamic acid carboxyl groups of the wall peptidoglycan. Beveridge and Koval (1981) proposed that the polar heads of the cell envelope phospholipids of E.coli were primarily responsible for its metal binding. For Bacillus licheniformis the predominant metal binding sites in the cell wall have been shown to be the techoic acids (Beveridge et al, 1982). For Rhizopus arrhizius the chitin of the cell wall has been implicated in uranium binding (Tsezos and Volesky, 1982 a.) and thorium binding (Tsezos and Volesky, 1982 b.).

Accumulation of metals by microorganisms is widespread and occurs by a variety of mechanisms. It is apparent the application of a biosorption process is more likely to be decontamination of metal-laden waste-water rather than metal recovery per se. To this end, much attention has been paid to accumulation of radioactive elements from solution. The biosorption of uranium (Zajic and Chiu, 1971; Horikoshi et al, 1981; Sakaguchi et al, 1978; Tuovinen and Dispirito, 1984; Tsezos and Volesky, 1981; Norberg and Persson, 1984; Jilek et al, 1978; Strandberg

et al, 1981; Nakajuma et al, 1982; Muzzarelli and Bregani, 1986; Yakubu and Dudeney, 1986; McCready and Lakshmanan, 1986; Friis and Meyers-Keith, 1986) and its application to uranium recovery has been widely researched. Biosorption of plutonium (Ruchloft, 1949) and radium (Tsezos and Keller, 1983) have also been investigated. However other, non-radioactive toxic metals also present great environmental hazards. Of these, cadmium is one of the most toxic and is the subject of this course of research. Consequently, the remainder of this review will be concerned with the toxicity and hazards posed by cadmium and work carried out on cadmium biosorption to date.

1.5 Biosorption of cadmium

1.5.1 Cadmium toxicity

Cadmium has long been recognised as highly toxic, but the effect of extended exposure to low concentrations of cadmium on human health was neglected until 1965 when cadmium was identified as the causative agent of itai-itai disease. The cadmium source was a base metal mining operation (Peterson and Alloway, 1979). It should be stressed that the levels of cadmium found in the local environment had been those previously considered as being low. Drinking water and rice were contaminated with paddy field soils containing

1-4 ppm of cadmium and rice containing 1 ppm. Itai-itai disease manifested itself in middle-aged or elderly people in the contaminated region as severe pain, skeletal deformation and proteinuria. These severe effects are subsequent to kidney damage which is responsible for Ca^{2+} loss proteinuria and glycosuria. The kidney is the critical organ with respect to cadmium toxicity (Lauwerys, 1979). Over the short term, cadmium exposure at higher levels leads to gastrointestinal malfunction with symptoms of abdominal cramps, headache, nausea, vomiting and diarrhoea. These effects are induced at levels of 15 ppm upwards (Lauwerys, 1979). Several studies have also been conducted on workers in alkaline battery producing factories where cadmium inhalation has been found to lead to kidney dysfunction. In one case the cadmium level within the factory was only 25 g/m^3 , but over a 10 year exposure period, the critical concentration of cadmium in the kidney was exceeded. A general consensus among several groups, including a W.H.O. review board, is that 100 g cadmium/g weight of kidney constitutes the critical level (Lauwerys, 1979).

The accumulation of cadmium as the causative agent of itai-itai disease demonstrates conclusively that direct occupational exposure to cadmium is not necessary to experience the effects of cadmium toxicity. Cadmium is

now known to accumulate in living organisms which can be particularly hazardous to man because of transfer through the food chain . A detailed survey of cadmium and its implications for environmental health can be found in the the review edited by Webb (1979).

1.5.2 Cadmium and the water industry

The problems associated with cadmium which confront water authorities have already been mentioned. A particular problem is the cadmium accumulation in sewage sludge which precludes cadmium sludge disposal to land (Pahren, 1979). As a consequence, where possible, U.K. water authorities usually dispose of sludge at sea.

The biomass produced in wastewater treatment plants already removes most of the heavy metals carried into the sewage treatment works. Primary sedimentation causes some elimination of heavy metals from the influent, but the bulk are removed during secondary treatment (Lester et al, 1979). However, this removal is not deliberate and the build up of toxic metals in sewage sludge creates problems in its own right. Thus water authorities would prefer to see the problem dealt with at source, that is at the factory of origin (Foster, 1983). Current metal recovery processes include electrolysis, ion exchange and neutralisation, carbonate and sulphide precipitation (Foster, 1983, Rahut, 1978). Several large companies have installed

such treatment plants to achieve the required discharge levels of cadmium in their effluent, but many smaller firms still continue to discharge without pretreatment, (Thompson and Prior, 1984). The reason for this is that operating and maintenance costs for physico-chemical treatment systems are prohibitively high. Larger firms find the extra expenditure difficult enough to deal with, but smaller firms appear unable to copy (Rahut, 1978; Thompson and Prior, 1984).

In conjunction with these disposal problems environmental concern about levels of cadmium discharged and disposal of cadmium bearing sewage sludge is rising. Limits set recently in the United States and by the E.E.C. are more stringent than the limits previously employed by U.K. Water Authorities (Thompson and Prior, 1984; Mance, 1984). Cadmium is on the European Community black list (List I), which comprises substances considered to pose the greatest environmental hazard (Mance, 1984). In the U.S.A. the cadmium discharge standards were revised in 1980. The revisions to E.E.C. standards are underway (Mance, 1984). Pressure in the U.S. and in E.E.C. countries has in many cases reduced cadmium use to those industries who absolutely require it, so that it is thought that the new legislation will not reduce the overall amount of cadmium used in industry much further (Rahut, 1978).

If all the aforementioned factors are considered, it is clear that cadmium removal from wastewater has a large potential market. A biotechnological process able to undercut the cost of presently employed methods of cadmium removal would be in demand. The pressure being exerted on trade waste producers and water authorities to cut back further on cadmium release into the environment is also likely to increase. In 1977 in a meeting between industrial users of cadmium and eight U.S. government agencies, a F.D.A. representative stated that sewage sludge disposed of to land and sea was the major source of cadmium entering the food chain in the U.S.A. (Plating and Surface Finishing, 1977). Metal contaminated sewage-sludge is presently disposed of in the North Sea by most U.K. water authorities. However, other E.E.C. countries now consider this to constitute an environmental hazard and have ceased dumping sewage sludge in the North Sea (Milne, 1987). Presumably the U.K. will eventually fall into line and as sewage sludge containing cadmium may no longer be sea dumped, the water authorities will need to recommend far stricter limits on the cadmium levels imposed on discharging industries. Furthermore, attempts to remove cadmium from sewage sludge have currently proved uneconomic (Thompson and Prior, 1984).

Hence, as limits on cadmium discharge levels are stringent and likely to increase, and as industry finds many of the present treatment processes expensive to operate, considerable scope exists for a more economic, perhaps biological, cadmium recovery process.

1.5.3 Cadmium uptake by microorganisms

Cadmium uptake by biomass has not received the scientific attention that uranium biosorption has, but the accumulation of cadmium by several microorganisms and some biopolymers has been investigated.

In studying the role of extracellular polymers in bioflocculation, Dugan and Pickrum (1972) followed up the work of Friedman and Dugan (1968) who demonstrated that polymer producing Zooglea ramigera 115 was more effective at zinc uptake than a non polymer producing strain. Dugan and Pickrum exposed Z. ramigera 115 flocs to a variety of metal ions including cadmium. After 18 hours exposure to cadmium (added as cadmium chloride) 31% of an initial cadmium concentration of 47.7 mg/l was removed (an uptake capacity of 0.19 mg Cd per gramme biomass). At an initial cadmium concentration of 23.7 mg/l, 62% of cadmium present was removed with Z. ramigera 115; again the specific binding capacity was 0.19 mg Cd per gramme biomass. When the bacterium was cultured on a medium less conducive to polymer production, the

specific binding capacity dropped to 0.01 mg Cd per gramme biomass. Dugan and Pickrum (1972) also investigated the binding capacities of a number of other floc-forming bacteria and found specific cadmium binding capacities ranging from 0.08 to 0.25 mg Cd per gramme biomass. The synthesis of extracellular polymer enhanced cadmium uptake from solution and was essential for flocculation by altering the water binding property of the polymers. Thus for flocculating bacteria metal removal is associated with flocculation.

While investigating the effect of extracellular polysaccharide production on copper and cadmium toxicity in Klebsiella aerogenes Bitton and Freihofer (1978) monitored the amount of cadmium bound by both an exo-polysaccharide producer and a non-producer. For K.aerogenes exopolysaccharide is produced as a capsule around the cell exterior. Bitton and Freihofer (1978) demonstrated that capsule formation enhanced cell survival and also determined the amount of cadmium removed from a 10mg/L solution after 30 minutes.

With initial polysaccharide concentrations of 82, 164 and 410 mg/l, the binding was respectively 9, 18 and 9% of cadmium present. The last result in the series may be erroneous as the proportion of cadmium removed from solution would be expected to increase with an increase in the amount of adsorbing material used. Bitton and Freihofer's own results for copper followed this trend. Horitsu et al (1979) isolated a cadmium tolerant pseudomonad, P.aeruginosa G-I which resisted cadmium and copper toxicity, but succumbed to chromium and mercury ions. Analysis of cadmium bound indicated that 88% of the cadmium was present in the cell cytoplasm and the remainder in the cell envelope. Further analysis indicated that cadmium was specifically bound to proteins and nucleic acids within the cytoplasm. In the cell envelope the majority of cadmium bound was associated with polyphosphate and polysaccharide. A pattern had begun to emerge for metal biosorption by pseudomonads. Previously Horitsu et al (1978) had determined that almost 80% of metal bound by a chromium tolerant strain P.ambigua G-I, to be associated with the cytoplasm. Subsequently Strandberg et al (1981) had observed that uranium bound by P.aeruginosa was predominantly associated with the cell interior and not a metabolic process. It would appear that metal binding by pseudomonads was associated predominantly with the cell interior.

E.coli is known to be quite cadmium tolerant (Mitra et al, 1975). Khazaeli et al (1981) developing previous experimental evidence (Mitra et al 1975) that suggested a high molecular weight cadmium binding component in E.coli (analogous in function to the metallothionins of mammals), tried to isolate the suspected cadmium binding protein. The detoxification strategy suspected to exist was isolation of toxic metal, i.e. compartmentalisation of the metal within the cell, as achieved by metallothionins in mammals. Thus cadmium accommodated E.coli cells are capable of binding cadmium without lethal effects. Over 90% of added cadmium (at 0.34 mg/l) was bound to accommodated cells. Of the cadmium bound to the cells, 80% was associated with the cytoplasm. A cadmium binding protein could be isolated from cadmium exposed cells, but not from unexposed cells.

The relationship between polymer production and cadmium binding by K.aerogenes was again investigated by Brown and Lester (1982). In continuous culture, increased polymer production by the cells again appeared correlated to enhanced metal uptake. However, Aiking et al (1982) cultured K.aerogenes cells under conditions of glucose limitation which precluded capsule formation and found evidence for an inducible mechanism for the non-

capsulate cells for tolerance to cadmium. Up to 67 mg/l cadmium was added to the culture medium for continuous K.aerogenes culture. This resulted in cessation of cell population growth and a lag of up to 5 hrs after which the culture exhibited greater resistance to cadmium toxicity than seen previously. These cells were capable of removing cadmium from the influent to achieve 2.4% of cell weight as bound cadmium. Subsequent investigation suggested that K.aerogenes possesses three mechanisms by which it may enhance its resistance to cadmium, the sulphide linked system Aiking et al (1982), a phosphate linked system Aiking et al (1984) and exopolysaccharide production, the latter being non-specific.

Gadd and Mowll (1983) investigated the relationship between cadmium uptake and toxicity for Saccharomyces cerevisiae noting that for many microorganisms toxicity is linked with metal uptake, in that many resistance mechanisms employed by microorganisms use the general strategy of reducing uptake of toxic metal. At initial concentrations of 1.04, 10.4 and 104 mg/l, the cadmium bound was equivalent to a 1.0, 4.5 and 35.7 mg cadmium per gramme biomass respectively. When glucose was included in the solutions, cadmium uptake was equivalent to 2.8, 8.5 and 31.7 mg cadmium per gramme biomass. Up to a certain concentration, cadmium uptake was glucose dependent to some degree. Loss of cell viability was greater as cadmium uptake increased, and presence of

glucose enhanced this effect at the two lower initial cadmium concentrations employed. Potassium release from the yeast cells was demonstrated to accompany cadmium uptake. Gadd and Mowll (1983) suggested that cadmium toxicity was manifested within the yeast cells as membrane disruption leading to release of potassium ions. The route of cadmium uptake was attributed to the calcium uptake mechanism of S.cerevisiae.

Rudd et al (1984) extracted the extracellular polymer (polysaccharide) produced by K.aerogenes and distinguished between the colloidal and soluble fractions of the polymer. The colloidal fraction was analogous to capsular polysaccharide. Rudd et al (1984) found that cadmium and copper tended to bind to the colloidal polymer and nickel to the soluble polymer when both polymer types were present together in the same metal solution. Adsorption isotherms indicated that metal uptake occurred beyond the level of initial cadmium complexation capacity which would suggest that more than one type of binding site was involved in metal complexation.

In 1984 details of two different approaches to a cadmium recovery system were published. MacCaskie and Dean (1984) prepared gel-packed columns containing immobilised cells of a Citrobacter sp. previously

isolated from lead-polluted soil. Cadmium solution (200 mg/l) was pumped through two test columns and 50% of influent cadmium was found to be retained in the columns. When the columns were joined in sequence, 70% of influent cadmium was removed. MacCaskie and Dean discovered that raising the temperature at which cells were cultivated prior to their inclusion in gel enhanced cadmium removed. This was attributed to the cadmium uptake mechanism being a precipitation whereby Citrobacter cells produced phosphate extracellularly via the action of a phosphatase on the cell surface; the phosphate thus produced precipitated out cadmium.

Citrobacter sp. exhibited increased phosphatase activity with increase in temperature; hence the removal of cadmium increased with increase growth temperature between 20 and 30°C. However, the temperature under which the recovery columns operated, in the range 10-30°C, had little effect on the cadmium removal efficiency of the columns. MacCaskie and Dean eluted cadmium from the packed columns by flushing them with citrate buffer. Subsequent cadmium removal by the columns showed a slight drop in efficiency during the second loading sequence, with 84.5% of the original removal capacity being attained. This system was capable of high efficiency cadmium removal and recovery was simplified because the cadmium phosphate

precipitated out and was retrieved as a white slurry. The major problem associated with the system was that the columns needed to be supplied with glycerol-2-phosphate in the influent to work at optimum efficiency.

Norberg and Persson (1984) devised a metal recovery system utilising Zooglea ramigera 115 as a biosorbent. After 20 hours culturing in a 3 l. fermenter, 17.5g/l of biomass was achieved of which 15g/l was determined to be polysaccharide. The biomass was then added to cadmium, copper and uranium solutions. The pH of the slurry was adjusted to achieve flocculation. Cadmium uptake from solution was found to decrease with decrease in pH (i.e. increasing acidity), and to reach equilibrium within 15 minutes of exposure. Cadmium uptake increased with culture age up to 8 days, but beyond this uptake declined. The optimum age of Z. ramigera 115 culture was 6-8 days for cadmium removal. Successive loading of the same biomass increased the amount of cadmium associated with biomass at each exposure to the same metal solution, but on the seventh sequence, the cadmium load of the biomass decreased.

Acid washing did not compromise the cadmium removal capacity of the biomass. Norberg and Persson (1984) achieved a specific binding capacity of 223 mg cadmium per gramme Z. ramigera 115 when a concentration of

0.66g/l biomass was added to a 490mg/l cadmium solution. They eluted cadmium from flocculated biomass by acid wash and proposed a cadmium recovery system whereby Z. ramigera was induced to flocculate, bind cadmium, release cadmium and be re-flocculated through control of pH in the bioreactor.

Sterritt and Lester (1986) have pointed out that this process gave an extremely efficient removal of cadmium (and other metal ions used) from solution, not necessarily because of Z. ramigera cells or the exopolysaccharide they produce, but because the process of flocculation involves metal ions (see Dugan and Pickrum 1972). In addition, the increased cadmium loading observed over successive exposure of the same biomass to the same cadmium solution does not signify that this biosorbent is particularly durable. Because the solution pH was raised each time biomass was added, it was pH change which favoured further cadmium uptake. Thus such a trial does not represent the change in metal removal efficiency that might be observed were fresh solutions employed during each phase of flocculation.

In 1985 the cadmium uptake capacity of a pseudomonad was examined and the influence of capsular polysaccharide formation on cadmium toxicity to K. aerogenes again investigated (Bauda and Block, 1985). Of two

K.aerogenes polysaccharide producing strains, the strain producing the greater amount of capsular polysaccharide was more resistant to cadmium toxicity. Bauda and Block (1985) proposed that cadmium binding by the pseudomonad they employed, P.fluorescens could be described by a Freundlich adsorption isotherm. However, a considerable amount of variation occurred in their data which was attributed to using cultures of different ages.

Ross (1986) used washed and detergent-treated mycelia of the fungi Penicillium spinulosum and Aspergillus niger to determine their copper, cadmium and zinc binding capacities. As pH decreased, cadmium binding also declined. Use of a metabolic inhibitor did not have a significant effect on cadmium binding and cadmium uptake was suggested to be a non-metabolic process. The adsorption isotherm determined fitted a Freundlich isotherm for P.spinulosum. As the initial cadmium concentration was increased, the proportion of cadmium removed from solution decreased for P.spinulosum. Treatment of the biomass with detergent enhanced its cadmium biosorption capacity, which was attributed to possible exposure of internal cell sites to metal through disruption of the biosorbent. Kiff and Little (1986) also used a fungus (Aspergillus oryzae) as a biosorbent for cadmium and also concluded that cadmium uptake was a non-active process. Fitting their data to

Langmuir and Freundlich models, they determined the Freundlich isotherm to give the best fit. The biosorbent was also immobilised within a column and cadmium sulphate passed through. the column was fully effective at cadmium removal during passage of the first 5l. of a 1mg/l cadmium sulphate solution, but declined thereafter as the biosorbent approached saturation.

Sterritt and Lester (1986) reviewed the uptake of cadmium, among other heavy metals, by activated sludge and determined that metal uptake could be described by a Langmuir adsorption isotherm. They noted the large uptake capacity achieved with Z. ramigera by Norberg and Persson (1984), the bacterium being a major constituent of most activated sludges, and stated that the full potential of the biosorbent was not realised. In activated sludge plants, the influent cadmium concentration is much lower than that used by Norberg and Persson (1984) and the cells already flocculated. The induction of flocculation was itself attributed partially responsible for the high cadmium removal they achieved.

1.6 Development of a cadmium recovery process using biomass

To date a number of both bacteria and fungi have had their capabilities as metal biosorbents assessed.

Recently some treatment systems for metal-bearing wastewater have been devised. In developing a cadmium biosorption process, several important criteria have therefore to be considered.

(i) Biosorbent

The material used for metal uptake needs to be cheap and reliable and should have a high metal binding capacity. In addition, the factors influencing metal uptake by the biosorbent and its mechanism of metal uptake are important and should be determined. The best uptake mechanism with regard to metal recovery is a non-metabolic process in which metal is firmly and rapidly bound to the biosorbent from which the metal may easily be recovered. Thus biosorption, as defined by Shumate and Strandberg (1985), which is similar to ion exchange, is likely to be the most useful mechanism of metal binding with regard to a metal recovery process. This mechanism is defined as a physical chemical interaction and as an adsorption process can be reversed to recover metal. Otherwise the adsorbed metal is firmly bound to the adsorbent and cannot be lost into the effluent from the treatment system. Precipitation of metal by some microorganisms occurs away from the cell which would be undesirable if metal precipitate were washed from the system. Ease of metal recovery when required is particularly important for cadmium as irreversible

permanent binding of the metal to biosorbent just presents another disposal problem. Therefore, if cells are employed as a biosorbent, extracellular and cell surface binding of cadmium are to be preferred to intracellular metal binding as the latter increases the difficulty of cadmium recovery. The use of dead biomass as biosorbent compared to live has several immediate advantages. Metal toxicity would not affect it, nutrients would not be required in the treatment process, and if one particular microbial strain were being employed because it had a high inherent metal binding capacity, a monoculture would not have to be maintained if the microorganism were dead when used as a biosorbent (Tsezos, 1986).

However, on the other hand use of live biomass as a biosorbent has a number of advantages which might make it a more desirable biosorbent than dead biomass. The great potential of microorganisms as metal recovery or removal materials is the low cost of production (i.e. growth) of the adsorbing material. One advantage that live biomass would possess is that, provided with inexpensive nutrients, a biosorption system could be self-regenerating with regard to biosorbent. However, it must be stated that this also raises a disposal problem. Live biomass, unlike dead biomass, could potentially in a continuous process adapt to the

conditions it was subjected to. Such adaptation might not be of benefit to the treatment process, though as reduction of susceptibility to toxic metals may occur through a reduction of net uptake of the metals. With live biomass presence of nutrients need not interfere with metal biosorption as the bioreactor could be purposely designed to ensure that growth of biosorbent is compartmentalised and the biosorbent then transferred continuously to another part of the bioreactor for metal removal and/or recovery. A dead biomass would need to maintain its biosorptive capacity over several treatment cycles to keep running costs low, whereas a live biomass could continually replace 'worn' biosorbent.

(ii) Bioreactor

As mentioned previously, the ideal site for a cadmium removal/recovery process is at the factory of origin which considerably reduces the volume of waste-water that needs to be treated. Ideally, the bioreactor should occupy as little space as possible if it is to be situated on a works premises. If the volume of waste-water to be treated is low (up to approximately 4000 litres per day) a fixed bed reactor might be employed. In many cases though, a larger volume might need to be decontaminated, in which case a fluidised bed would be employed. The bioreactor would need to be

designed to remove continuously and also, if required, recover metal. Thus for fixed or fluidised beds a bioreactor consisting of at least one column in series with another might be envisaged. Furthermore, placing columns in series would allow removal of metal to approach a theoretical maximum of 100%, although in practice this may not be necessary. With live biomass in particular a series of columns would be required as the regeneration of biomass would need to be compartmentalised in order to prevent nutrients interfering with biosorption.

In summary, the mechanism of metal removal by the biosorbent and environmental factors affecting it need to be considered. Bound metal should be recovered easily and cheaply and the cost of producing (and preparing, if necessary) the biosorbent kept to a minimum. The bioreactors used needs to be capable of treating the volumes of wastewater anticipated without occupying too great a space, and ideally should be simple to run and require little maintenance.

1.7 Objectives

Polysaccharide production by cells is known to enhance cell survival in the presence of toxic metals. The bacterial strains selected for use as potential biosorbent in this course of investigation included the

polysaccharide producers K.aerogenes and Arthrobacter viscosus. The latter produces a loosely associated soluble extracellular polysaccharide (Gasdorf et al, 1965), the structure of which is known (Sloneker et al, 1968). The former produces exopolysaccharide that is bound around the cell in the form of a capsule. Throughout the course of investigation, live biomass was used (except where performance of dead biomass was assessed for the sake of comparison) as the proposed conclusion to the research program was use of live biomass to recover cadmium in a fluidised bed and assess the degree of success attained with such a process. The development of most biosorbent utilising metal removal/recovery systems appears to be toward use of dead biomass, mainly owing to its convenience, but it was felt that because of some of the potential attributes of live biomass, such a system, despite the acknowledged drawbacks, needed to be properly assessed. For comparison Arthrobacter and Klebsiella were employed which were not capable of polysaccharide formation. Arthrobacter and Kaerogenes were selected because the production of extracellular polymer was considered likely to enhance their cadmium binding capacity..

In addition, Arthrobacter sp. were selected as they are generally robust (Keddie and Jones, 1983) and also

because Arthrobacter species have been found in areas rich in uranium and might thus be inferred to possess some resistance to metal toxicity. Arthrobacter species also alter morphology during their life cycle and the influence of this on biosorption was of interest. Kaerogenes was selected as it possesses an inducible resistance mechanism to cadmium (Aiking et al, 1982) and its extracellular polymer known to be capable of metal binding.

Two pseudomonads, P.cruciviae and P.putida were also selected as their uptake of cadmium was anticipated to be intracellular, and the effect of this on cell survival and cadmium recovery was of interest.

Once the biosorptive capacity of these bacteria had been determined, the environmental factors affecting cadmium uptake were to be assessed in conjunction with removal of cadmium by live biomass in a fluidised bed.

2. Materials and Methods.

2. Materials and Methods

2.1 Methods

2.1.1. Metal analysis

Metal content of solutions was determined by differential pulse polarography. The concentration of an ionic species in solution is proportional to the current induced by applying a potential difference between two electrodes placed in the solution. This is the basis of polarography of which differential pulse is an advanced technique which generates a signal in the form of a peak, the height of which is proportional to the concentration of an ionic species in solution. This method can detect several metals (including cadmium) down to levels of 0.5mg/l. Certain metals can be detected to 1ppb if anodic stripping voltammetry, a similar technique, is used(Ryan, 1984).

The polarographic equipment used for metal analysis was purchased from EDT Research and included the Model ECP 100 polarograph, electrodes and ECP 102 stand (Photograph 1). Peak traces were recorded on a chart recorder. Samples were degassed in the electrochemical cell of the ECP 102 stand by nitrogen to displace oxygen from solution. Solutions were all degassed for 5 minutes prior to polarography if over 25mg/l. Solutions of less than 25mg/l were degassed for 10 minutes. Biomass was added to standard 100ml solutions of metals

for 5 minutes unless otherwise stated, then a sample extracted from solution flask and centrifuged. The supernatant was analysed for metal content in the polarographic cell following degassing and addition of an electrolyte. The metal concentration of the initial solution was measured and the metal removed by biomass determined by obtaining the difference in concentration of metal between the pre-exposed and post-exposed solutions. Differential pulse mode was always set with a drop time of 1 second.

(i) Polarography electrolyte

The same amount of concentrated electrolyte was added to metal solutions to be analysed, but the standard amount added was 0.1ml to 10mls sample (i.e. 1/100 dilution). The electrolyte composition was 32.68g sodium acetate plus 1.3mls glacial acetic acid made up to 100mls with deionised water. For experiments determining pH effect on biosorption, the electrolyte was prepared without 1.3mls glacial acetic acid.

(ii) Glassware

As glassware is capable of adsorbing metal ions, all glassware used was treated with Dimethyldichlorosilane (obtained from BDH Ltd., Poole) to make it non-adsorbent. Experimentation showed that treated glassware did not remove any metal from solution.

(iii) Standard metal solutions

All standard metal solutions were prepared with deionised water and stored in treated bottles. Metal salts used were all 'Analar' standard.

2.1.2 Culture media and culture conditions

The standard culture medium used was composed of the following:

D-Glucose	10g/l
Bacteriological peptone	5g/l
Casein Hydrosylate	3g/l
Yeast Extract	3g/l
Magnesium sulphate heptahydrate	1g/l

Standard incubation time was 48 hours from time of inoculation. Cultures were incubated as 100ml aliquots of medium in 250ml flasks in an incubated rotary shaker at 25°C and 150rpm. Cells were harvested by centrifugation in a centrifuge at 2,500g for 30 mins; supernatant was discarded and the cells washed in quarter strength Ringers solution, then re-centrifuged, washed again and stored in a volumetric flask in 1/4 strength Ringers solution. Cells were exposed to metal solution within 4 hours of harvesting.

2.1.3 Dry cell weight determinations

Cell suspensions in volumetric flasks had samples withdrawn following harvesting, and at least five samples were taken for the dry cell weight present in suspension to be determined. The known volume samples were air dried at 105°C, stored in a dessicator until cooled, and the average dry weight of the cells calculated.

Unless stated, 30mg of biomass was added to metal solutions by withdrawing the appropriate volume from stirred suspension and centrifuging in an MSE Microcentaur for 5 mins (unless stated) at 11,600g. Supernatant was discarded and the biomass pellet added to the metal solution in a chemical flask and swirled vigorously for approximately 30 seconds to mix the contents, then left to stand for ten minutes. Metal solutions were unbuffered to avoid ionic interference apart from any that might be intended. pH as standard was 7.5 and temperature 20°C. This represented standard procedure which was always followed without modification, unless otherwise stated.

2.1.4 Effect of temperature on biosorption

Biomass was added to cadmium solutions maintained at 20, 40 and 50°C. Samples were taken after standard exposure time of five minutes.

2.1.5 Kinetics experiments

Samples were taken and centrifuged for only 30 seconds at 11,600g before transferring to a holding container for later analysis. The short centrifugation period was employed to allow the most immediate analysis possible. Subsequent investigation determined that this period of centrifugation was sufficient to separate cells from solution at 11,600g and produce results within a 95% confidence interval of those obtained at the normal centrifugation time of ten minutes. It should be stressed that the object was to obtain as immediate a sample as possible and each sample was subjected to the same 30 second centrifugation period.

2.1.6 Effect of pH on cadmium biosorption

Polarography electrolyte used for addition to these samples was composed of 32-68g sodium acetate made up to 100mls with distilled water. Solutions of the appropriate pH were prepared with buffer and drop addition of concentrated hydrochloric acid.

2.1.7 Cadmium uptake by different amounts of biomass

A suspension of biomass was prepared as standard and the appropriate volumes removed and centrifuged to give a range of biomasses from 2mg to 180mg for A.viscosus, P.cruciviae and P.putida . Biomass was exposed to cadmium at 1mg/l, a low concentration being used to try

to achieve total removal of available cadmium by biomass at the highest biomass levels used.

2.1.8 Cadmium uptake by live, autoclaved, disrupted and formaldehyde-treated biomass

Biomass was harvested, washed and resuspended in a known volume of Ringers solution as standard in four separate containers. One sample was subjected to autoclaving at 120°C for 15 minutes; another exposed to 10% formaldehyde for 30 minutes; the third biomass sample was left in an ultrasonic bath for 30 minutes, both to kill and disrupt the cells. Microscope examination of this sample at x1000 confirmed that cells were disrupted.

2.1.9 Effect of cadmium biosorption on cell viability

The viable count was determined for different bacterial species prior to addition of cells to cadmium solution (100mg/l initial cadmium), after cadmium biosorption (10 minutes exposure) and after acid desorption (15 minutes exposure to 0.1M hydrochloric acid). As centrifugation was carried out at 11,600g for 10 minutes, the exposure times stated could also be viewed as being 20 and 25 minutes respectively through biosorption and desorption as the stated times are time elapsed between biomass addition to cadmium solution and acid and transfer to centrifuge for full cell recovery. Ten-minute

centrifugation at this rotor speed had been determined to achieve cell recovery without loss of biomass, as no viable colony forming units were recovered from supernatant following centrifugation. Between each stage biomass was returned to exactly the same volume of solution that it had previously occupied. Fifty mls of solution was employed to reduce the amount of centrifugation necessary. Viable counts were made after 48 hours incubation on Oxoid nutrient agar.

2.1.10 Effect of polysaccharide on cadmium biosorption

The actual polysaccharide constituents of serotype 8 exopolysaccharide (produced by K.aerogenes 9128 and 9527) are known, as is the structure of A.viscosus 9728 polysaccharide (Slonecker et al, 1968). The cadmium binding in solution of these monomers (D-glucuronic acid, D-glucose and D-galactose for K.aerogenes; D-mannuronic acid, D-glucose and D-galactose for A.viscosus) was determined by adding 30mg of each monomer into the electrolytic cell of the polarograph containing a solution containing a known concentration of cadmium. The solution was briefly stirred with a glass rod and the cadmium present in solution in the cell then monitored over ten minutes. In addition to the cadmium removal of each separate monomer, 10mg each of the three monomers of K.aerogenes and A.viscosus exopolysaccharide was added to cadmium

solution. Polysaccharide was recovered from A.viscosus and the binding capacity of 29mg purified polysaccharide compared to that of 29mg A.viscosus cells. The cadmium binding capacity of both wet and dehydrated polysaccharide was also determined.

Polysaccharide was recovered from A.viscosus using 100mls culture fluid diluted 1:4 with water, to which 0.5 volume ethanol was added. This mixture was then centrifuged at 2,500g for 120 minutes. The supernatant was decanted off and polysaccharide precipitated by addition of 1g KCl per 100ml solution and 2.5 volumes of ethanol. The precipitate was then redissolved in deionised water (50ml) and re-centrifuged in a Microcentaur centrifuge at 11,600g for 30 minutes and reprecipitated as previously. A portion of the precipitate was then abstracted for dehydration (air dried at 105°C) and the remainder mixed with 5ml distilled water from which a sample was taken for a dry matter content determination. 29mg of dehydrated polysaccharide was eventually recovered and its cadmium uptake determined. An appropriate amount of 'wet' polysaccharide (i.e. equivalent to 29mg) was added to cadmium solution and its biosorptive capacity determined. To determine whether or not any polysaccharide was 'carried over' or produced by A.viscosus cells under standard biosorption trial

conditions, 30mg of A.viscosus were added to 10ml deionised water, swirled and left to stand for 5 minutes. The suspension was then centrifuged at 11,600rpm for 10 minutes following which the supernatant was decanted off. Four volumes of ethanol and 0.5g KCl were added to precipitate any polysaccharide. A small clump of polysaccharide was thus precipitated which was redissolved in 10ml water, re-centrifuged and again precipitated. This small precipitate was carefully removed by pipette and added to cadmium solution (10ml of 2mg/l Cd^{2+}). The cadmium removal from solution was monitored and then the polysaccharide dry matter content determined by air drying overnight (10 hrs) and allowance made for the amount of cadmium bound (i.e. this was subtracted from the overall dry weight). This procedure was then repeated with 30mg of A.viscosus cells from the same culture.

2.1.11 Effect of presence of other metal ions on cadmium biosorption

A series of experiments was conducted with biomass provided by A.viscosus, K.aerogenes 9128 and A.globiformis biomass to determine the influence of other divalent heavy metal cations on the amount of cadmium bound. Metal uptake from 100ml solutions at 20°C and pH 7 ± 0.5 was determined for individual 1 and 10mg/l solutions of Cd^{2+} , Pb^{2+} , Ni^{2+} , Ca^{2+} and Zn^{2+}

with 30 mg biomass. The uptake of cadmium from mixed solutions was then determined for 30mg of each aforementioned bacterial species. These solutions were all 100ml in volume and had the following compositions:

- I 0.33mg/l each of Cu^{2+} , Zn^{2+} and Cd^{2+}
- II 1.0mg/l each of Cu^{2+} , Zn^{2+} and Cd^{2+}
- III 10mg/l each of Cu^{2+} , Zn^{2+} and Cd^{2+}
- IV 0.5mg/l each of Cd^{2+} and Ni^{2+}
- V 10mg/l each of Cu^{2+} , Zn^{2+} , Ni^{2+} , Pb^{2+} and Cd^{2+}

2.1.12 Effect of growth medium and culture age on cadmium biosorption

Growth medium sufficient for 100ml was made up in 20ml distilled water, then mixed into 80ml of cadmium solution of which controls had been prepared to determine the cadmium concentration after 20ml addition. Thus the amount of cadmium bound by media constituents was determined at 21 and 41mg/l cadmium for A.viscosus and A.globiformis.

Arthrobacter species undergo morphological change during the course of their growth cycle (Keddie and Jones, 1983). To investigate if this produced any major changes during the growth cycle, five 250ml flasks containing 100ml of standard growth medium were inoculated with 1ml inoculum from a stationary phase A.viscosus culture and microscope slides made and biomass harvested from each at 24, 48, 72, 96 and 120 hours culture time respectively. For each sample, microscope slide smears were Gram-stained and examined to ascertain the balance of the cell population (i.e the approximate proportion of cocci to rods). At these time intervals 30mg of biomass was prepared and used as a biosorbent in 10mg/l cadmium solutions.

2.1.13 Electron microscopy

Transmission electron micrographs and energy-dispersive

x-ray data to identify elements present within cells were obtained. Cadmium exposed cells were dehydrated in gluteraldehyde, stained with 1% Pb/OS and then embedded in plastic. Non-stained samples were also included, primarily for x-ray examination. Samples were also carbon-coated for extra stability under x-ray examination. The transmission electron microscope employed was a Joel (Ltd) Model 2000fx; the x-ray detector a Lisk Co. Detector.

2.1.14 Efficiency of cadmium elution from biomass by several chemical desorption agents

The following reagents were prepared for use as desorption agents:

- i Sulphuric acid (0.1M)*
- ii Nitric acid (0.1M)*
- iii Hydrochloric acid (0.1M)*
- iv Citric acid (0.1M)
- v Sodium hydrogen carbonate (0.1M)*
- vi Sodium carbonate (0.1M)*
- vii Sodium acetate (0.1M)
- vii E.D.T.A. (0.1M)

For each desorption agent, 30mg of A.viscosus (preloaded with cadmium after 10 minutes exposure to 15ml of 1mg/l cadmium solution) was added and the amount of cadmium displaced from the biomass into solution assessed. Desorption agents marked with an asterisk were also used

with 50mg of P.putida and 25ml of cadmium solution (1mg/l). Once desorption was achieved, the biomass was then reloaded in the appropriate volume of 1mg/l cadmium solution and subsequently again desorbed to enable its efficiency of biosorption and desorption to be determined over two biosorption/desorption cycles.

Low volumes of cadmium solution and desorption agent were used to reduce the amount of centrifugation required.

2.1.15 Desorption kinetics

Procedure was the same as that for biosorption kinetics studies. Biomass was preloaded with 5mg/l cadmium solution prior to elution.

2.1.16 Effect of cadmium concentration in biosorption phase on subsequent desorption

Biomass was preloaded in cadmium solutions of different concentrations and then the cadmium bound eluted by 0.1M hydrochloric acid. In a complementary study 30.5, 61.5 and 82.0mg of A.viscosus was continuously loaded in 100ml 10mg/l cadmium solution over ten loading (biosorption) cycles and the amount of cadmium removed from each solution by the same biomass assessed.

2.2 Cadmium removal from solution by biomass in a

fluidised bed

2.2.1 Growth and cadmium adsorption in a fluidised bed by A.viscosus

Inoculum (1.0l) was prepared from exponential phase culture (as determined from spectrophotometry of culture) to inoculate bed I (Figure I) which contained 19l of sterile medium. Circulation was initiated and air flow maintained at 8.0l/min \pm 2.0 and the flow rate of the culture was 1.5 gallons/min. 10ml of antifoam compound (BDH; silicon type A) was added to the reservoir every 24 hours. Support material in the perspex column was chosen due to its wide availability, low cost and low adsorption. Sand had been sieved to provide particles of 1.18mm diameter which was then autoclaved and air dried at 100°C. After cooling 1kg of dry sand was added to the biosorption column prior to addition of any liquid to the system. Support was retained between two stainless steel meshes of 1.0mm pore size. A distributor was built into the bed I system under the biosorption column to ensure even air flow through the column.

The pH dissolved oxygen concentration and temperature within the biosorption column were noted over a period of ten days. Samples of sand with attached biomass were taken at 22, 48, 72, 96, 116.5, 149 and 197.5 hours

after inoculation and the amount of biomass present on the sand and the biosorption capacity of the sand associated biomass determined as follows:

(i) Cadmium uptake

Sand biomass was gently water washed, excess water decanted and the sand and biomass added to 12.5 and 25.0mg/l cadmium solutions (100ml) and cadmium uptake determined.

(ii) Sand associated biomass

Cadmium exposed biomass was subjected to 30 minutes ultrasonic vibration, washed vigorously and the wash water air dried to determine the amount of biomass attached to the sand.

Samples for microscope examination, and spread plates (nutrient agar, Oxoid) were also taken from the fluidised bed at intervals to determine cell numbers within the circulating medium and identify presence of any contaminants (the system was open).

2.2.2 Uptake of cadmium by A.viscosus biomass within fluidised bed I

A number of experiments were carried where the uptake of cadmium by biomass within the fluidised bed was assessed.

(i) Control experiments

(a) Circulation of 25mg/l cadmium (total volume of solution 20.0l) around bed I and the amount of cadmium bound to bed components and 1kg sand assessed.

(b) Circulation of 50mg/l cadmium (total volume 20.0l) around bed I and the amount of cadmium bound when silicon antifoam oil (50ml) and subsequently, enough dry nutrient media to 20.0l capacity for standard medium was assessed.

(c) A.viscosus without medium

(ii) 24 hour culture of A.viscosus (19.0l medium inoculated with 1.0l cells). Bed I drained of nutrients after 24 hours and distilled water circulated within the system to flush out excess nutrients. Washwater drained, 20.0l cadmium at 50mg/l prepared in the reservoir, circulated through the bed for 1 hour and cadmium removal monitored by samples taken from the bed in non-adsorbent glassware. Following the trial the amount of biomass in the circulating fluid and attached was determined as previously.

2.2.3 Growth of A.viscosus in fluidised bed II:

attachment of A.viscosus biomass to support.

Bed II was composed of glass QVF fittings (Figure 2). Trials carried out on Bed II were concerned solely with the attrition of support attached biomass within the fluidised bed. However, the amount of cadmium actually bound by bed components was assessed to compare uptake of components in a glass bed compared with a bed composed of A.B.S. and perspex (Beds I and III). 15.0l of cadmium solution at 50mg/l was prepared within the newly constructed bed and circulated at 5.0l/min. Cadmium concentration within solution was monitored over 16 hours. A number of different support materials were selected for use. In each case the following procedure was adopted:

(i) 1.0kg of support material autoclaved and added to Bed II

(ii) Sterile medium (14.0l) added to bed plus 1.0l inoculum A.viscosus and circulated at 5.0l/min for 24 hours

(iii) Medium drained and system gently flushed with 15.0l distilled water for 30 minutes at 1.0l/min. Wash water then drained and dry matter determination carried out for the wash water.

(iv) 15.0l cadmium solution (made up to 50mg/l Cd^{2+}) was circulated within the bed for 60 minutes then drained. Dry matter content was again assessed.

(v) 15.0l deionised water made up to 0.1M hydrochloric acid then circulated for 60 minutes, bed drained and dry matter content of the effluent assessed.

(vi) The support was then removed from the biosorption column and placed in an ultrasonic bath for 30 minutes to remove any biomass. The support was vigorously shaken in the water present, then drained and rinsed. both initial drain water and rinse water were air dried at 105°C to determine dry matter content.

The assumption was then made (in practice not realisable) that all biomass was now stripped from the system and the overall weight from each run designated 100% so that the amount of dry matter (i.e. biomass) lost into each liquid phase could be assessed and compared for different support materials.

The support materials used were:

(A) Sand grains of 1.18mm diameter

(B) Builders Gravel

The dark grey gravel was initially found to turn circulating fluid black. As a result, acid leached

gravel (gravel left in 0.1M sulphuric acid for 8 hours) was used in the trial, but in the desorption phase circulating fluid still began to turn brown/grey.

(C) Viton tubing

Viton tubing of 1mm internal diameter was cut into lengths of 4 ± 1 mm

(D) Perspex tubing

Internal diameter 4mm, external diameter 6mm; length 6 ± 1 mm.

(E) Glass beads

Of 2mm diameter

(F) Ceramic beads

Porous ceramic beads (as used in horticulture) of 5 ± 1 mm diameter. (Note that between trials in any of the fluidised beds the beds were cleaned - see section 2.2.5).

2.2.4 Cadmium biosorption by K.aerogenes 9128 within fluidised bed III

Fluidised bed III (Photograph 2) was built predominantly from A.B.S. 'plastic'. Two columns were present in the bed to provide a system wherein cadmium might be accumulated by biomass within one column whilst the other maintained or renewed biosorbent on support simultaneously. The ultimate goal of the system was to achieve a continuous process but this was not achieved within the time available. 1kg of ceramic beads was

prepared as support material. 14.0l of sterile culture medium in the bed was inoculated with 1.0l of K.aerogenes 9128 and the medium circulated within the bed for 48 hours at 5.0l/min.

Medium was then drained from the system, 15.0l deionised water added and circulated at 1.0l/min for 30 minutes. This wash water was then drained and the dry matter content assessed. 15.0l of 50mg/l cadmium solution was then circulated within the fluidised bed for 60 minutes following which it was drained and the dry matter content assessed. Support material was then removed to the column and its dry matter content assessed as per previous method.

The trial was repeated twice and the average biosorption over 60 minutes determined. In addition, the proportion of biomass retained on the support material was determined.

2.2.5 Fluidised bed cleaning regime

On conclusion of a trial, fluidised beds were cleaned as follows:

- (1) Flushed with water at 10l/min (2 hours). Drain.
- (2) Flushed with 10% Lysol (to disinfect) at 5l/min (2 hours). Drain.

(3) Flush with water (10l/min for 2 hours). Drain.

(4) Acid cleaned with 0.1M HNO_3 , 0.1M HCl or 0.1M H_2SO_4 to leach out any metal ions. (4 hours at 2l/min).

Drain.

(5) Flush with water (2 hours at 10l/min). Drain.

Assess dry matter content. If more than 30mg/l present, repeat process.

2.3 Materials

2.3.1 Bacterial cultures

Bacterial cultures were obtained from the National Collection of Industrial and Marine Bacteria (N.C.I.M.B.), Torry Research Station, Aberdeen. The strains used were:

- (1) Arthrobacter viscosus NCIMB 9728
- (2) Arthrobacter globiformis NCIMB 8605
- (3) Klebsiella aerogenes NCIMB 9128
- (4) Klebsiella aerogenes NCIMB 9527
- (5) Klebsiella aerogenes NCIMB 9528
- (6) Pseudomonas putida NCIMB 8858
- (7) Pseudomonas cruciviae NCIMB 9432

2.3.2 Biochemicals

Microbiological media were obtained from Oxoid, Wade Road, Basingstoke, Hampshire.

D-mannuronic acid and D-Glucuronic acid-1-phosphate were obtained from Sigma Chemicals Ltd, Fancy Road, Poole, Dorset.

D-Glucose was obtained from BDH Ltd, Poole, Dorset.

2.3.3 Chemicals

Chemicals were 'Analar' grade, obtained from BDH Ltd.

These included:

Mercury (required for the polarography)

Metal salts - the metal preparations were made with the following salts:

Cd^{2+} from $\text{CdCl}_2 \cdot 1/2 \text{H}_2\text{O}$

Zn^{2+} from $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$

Pb^{2+} from $\text{Pb}(\text{NO}_3)_2$

Ni^{2+} from $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$

Cu^{2+} from $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$

KEY: Photograph 1. Polarograph

(1) ECP 100 Polarograph

Control unit .

(2) ECP 102 Polarographic stand

Incorporating electrochemical cell, mercury
dropping electrode, reference electrode and
standard electrode and N₂ gas control

(3) J-J. Chart Recorder

KEY: Figure 1. Fluidised Bed 1.

- (1) Pump
- (2) Reservoir
- (3) Rotameter
- (4) Distributor
- (5) Perspex biosorption column
- (6) Control valve

T: Thermocouple

S₁, S₂, S₃... Sampling ports

D.O.: Dissolved oxygen probes

pH: pH probe

a: Aeration points (arrows indicate flow direction)

m: stainless steel mesh (all of 1.0mm pore size)

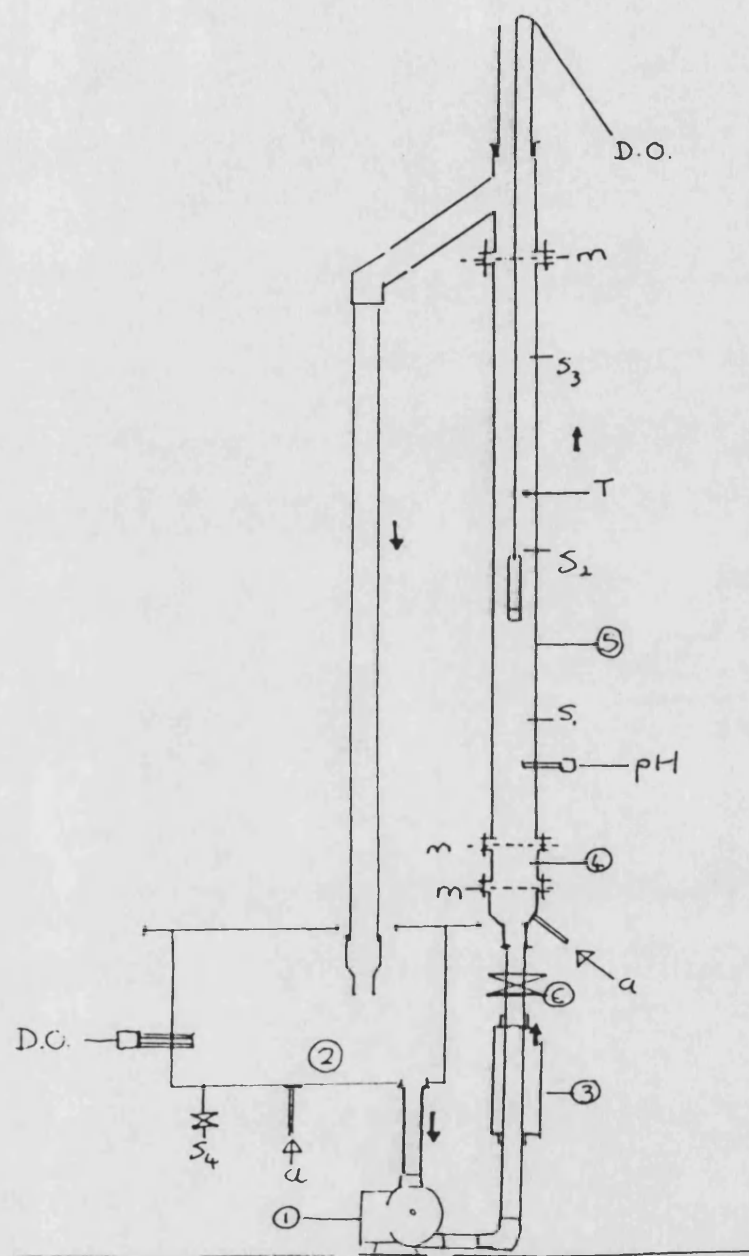
Perspex main column of bed 2.0m in length and 0.05m internal diameter.

Distributor placed at bottom of column to prevent 'slugs' of air (i.e. large bubbles) entering column. Distributor contents were 2.0mm diameter glass beads.

All fittings, apart from column and tank were composed of A.B.S. 'plastic'.

Working capacity: 20.0L

FIG.1 FLUIDISED BED1.



KEY: Figure 2. Fluidised Bed II.

- (1) Pump
- (2) Perforated airline in reservoir
- (3) Rotameter
- (4) Distributor
- (5) Glass biosorption column (2.0m long, 7cm i.d.)
- (6) Reservoir

T: Thermocouple

dD.O. : Dissolved oxygen probe

S_1, S_2, S_3 Sampling ports

pH: pH electrode

V_1, V_2 : Control (of flow)

Valves: V_1 - up flow valve

V_2 - by pass valve

D: Drain valve

R: Relief valves (for draining bed)

$R_1; R_2$ = pressure of relief valve

Black arrows show direction of liquid flow.

White arrows indicate air injection

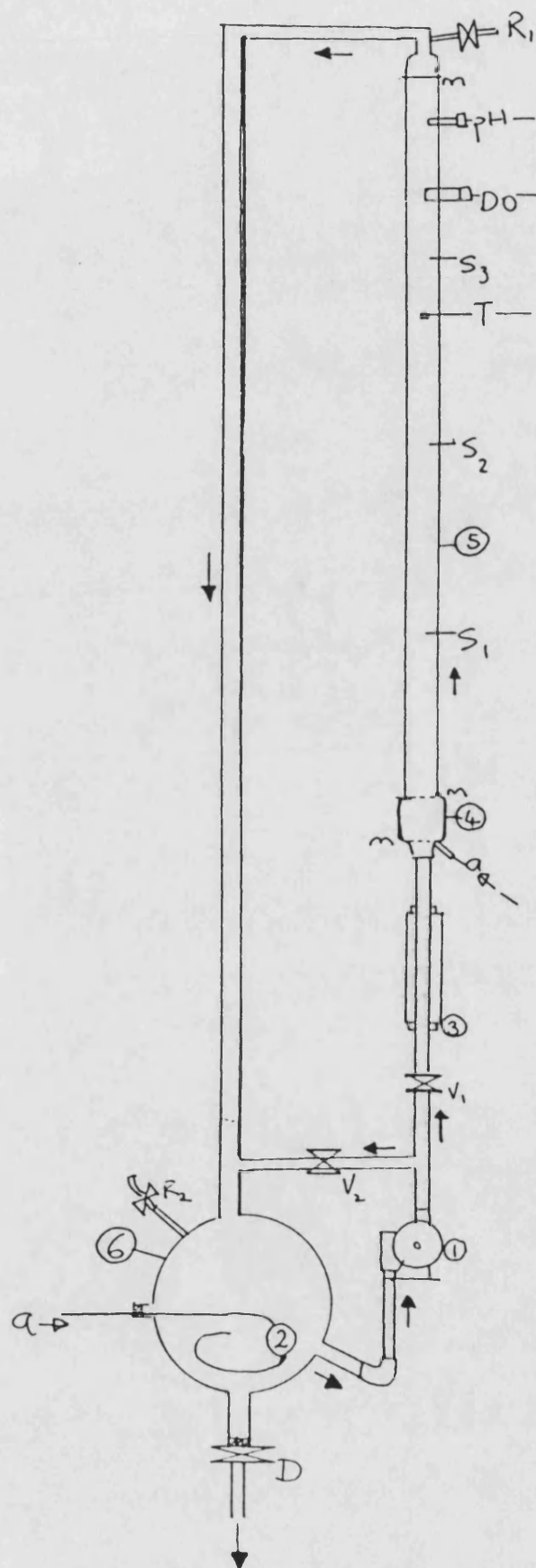
Distributor used to give even air distribution through column. Contents of distributor were 2.0mm diameter glass beads.

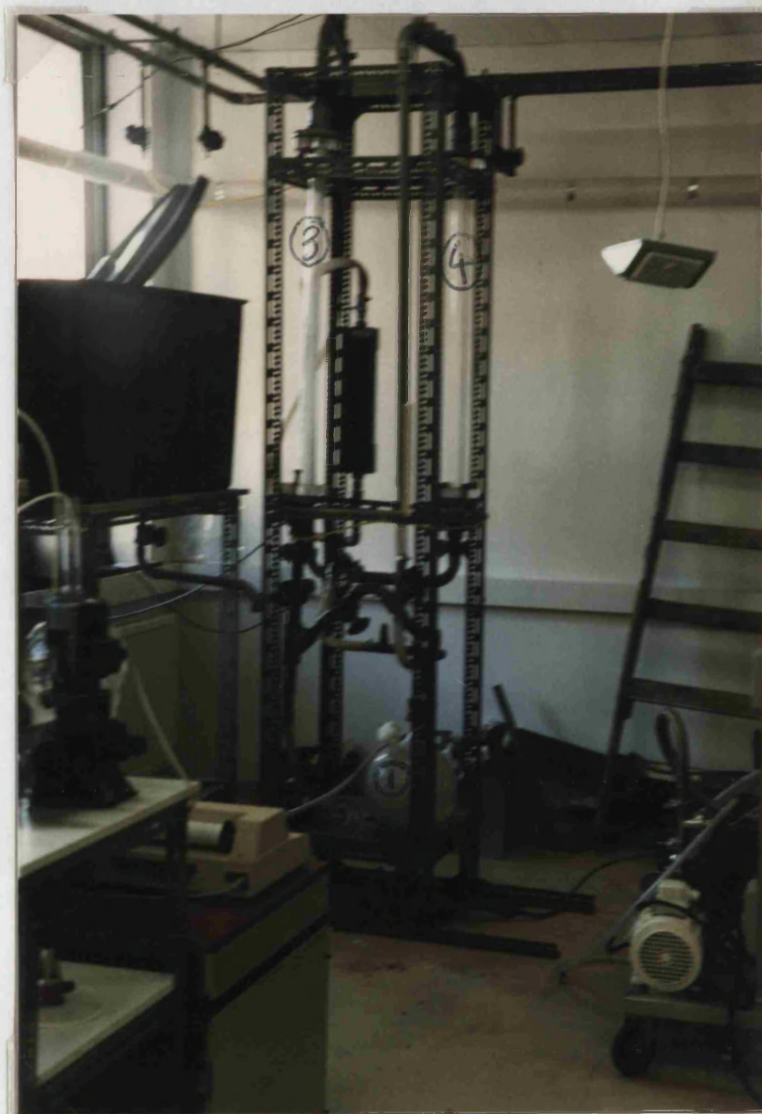
'm' steel mesh; 1.0mm pore size.

Composed of QVF glassware and associated fittings.

Working capacity: 15.0L

FIG.2 FLUIDISED BED 2.





Photograph 2.

KEY: Photograph 2: Fluidised bed III

(1) Reservoir

(2) Pump

(3) and (4) Perspex biosorption columns; one to act as a feed column. Feed column to be used for culture maintenance while biosorption achieved in second column.

Working volume: 15.0L (20.0L if second column in use)

Temperature, dissolved oxygen content and pH within system monitored by probes.

Distributors present at base of each biosorption column containing 2.0mm diameter glass beads. Steel meshes in system of 1.0mm pore size. All fittings A.B.S. 'plastic'.

3.Results and Discussion.

3. Results and Discussion

3.0 Results

The experimental work was carried out to achieve two broad aims. Firstly to determine the cadmium adsorption potential of several viable bacterial strains, specifically of those capable of producing extracellular polysaccharide, and investigate the influence of environmental and other factors on both cadmium biosorption; and also efficiency of cadmium recovery, i.e. desorption. This constituted the bulk of the experimental work. Secondly, the remainder of the experimental work as described in the last section of this chapter was concerned with trials of cadmium biosorption on a larger scale, by biomass associated with support particles in a fluidised bed; to determine the feasibility of using live biomass in such a reactor.

3.1.0 Cadmium biosorption

3.1.1 Kinetics of cadmium biosorption

The rate of cadmium uptake by the bacterial species used was monitored for at least 60 minutes. From the typical results presented (figures 3, 4 and 5) it can be seen that in each case 90% of the equilibrium uptake concentration of cadmium was reached within five

FIG.3 UPTAKE KINETICS FOR 1mg/L CADMIUM SOLUTION
BY 30mg PS.PUTIDA AND 30mg PS.CRUCIVIAE.

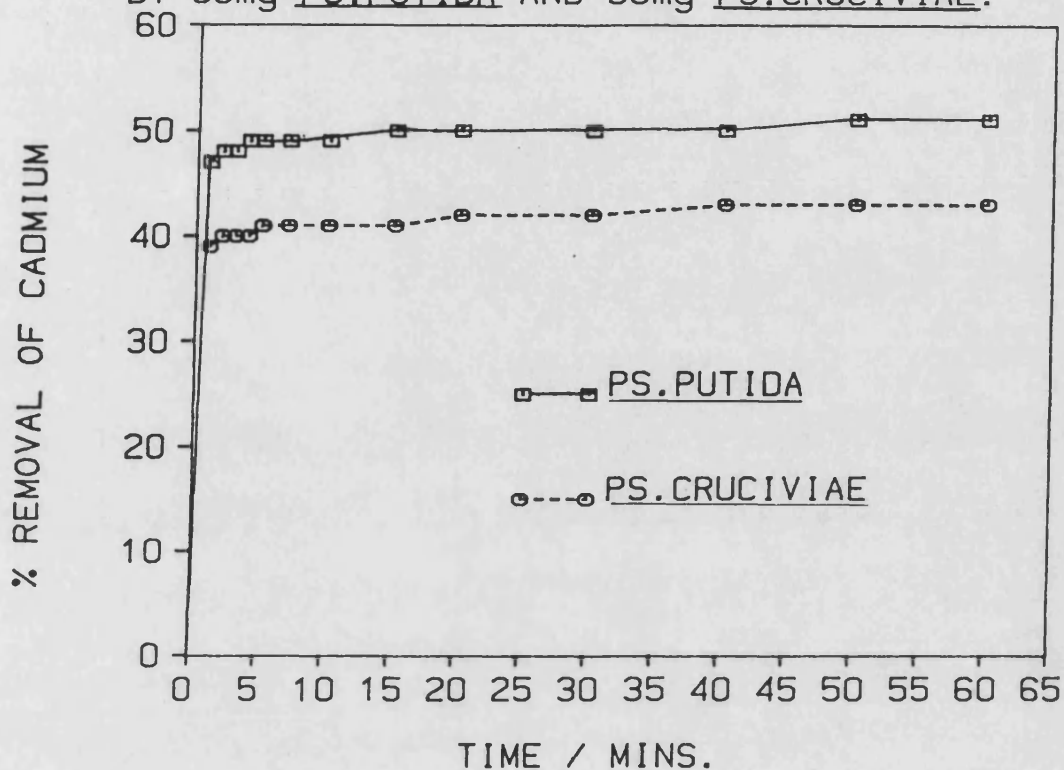


FIG.4 UPTAKE KINETICS FOR 1mg/L CADMIUM SOLUTION
BY 30mg A.VISCOSUS AND 30mg A.GLOBIFORMIS.

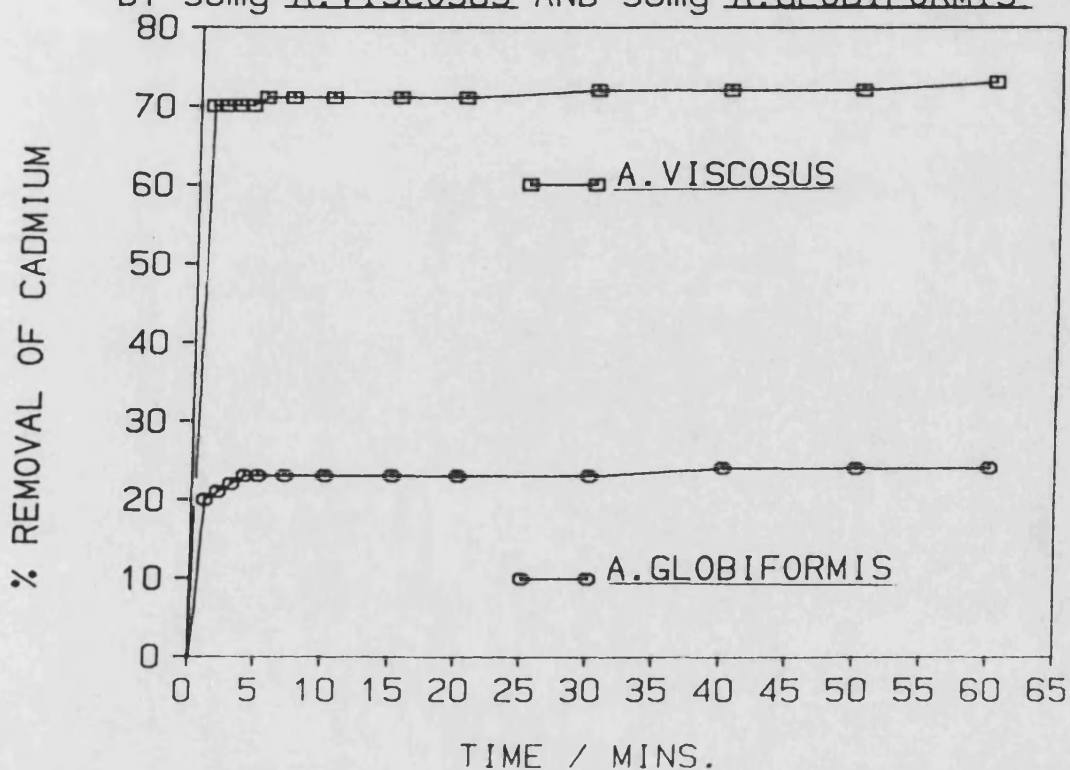


FIG.5A UPTAKE KINETICS FOR 10mg/L CADMIUM SOLUTION
BY 30mg KLEBSIELLA AEROGENES STRAIN 9128

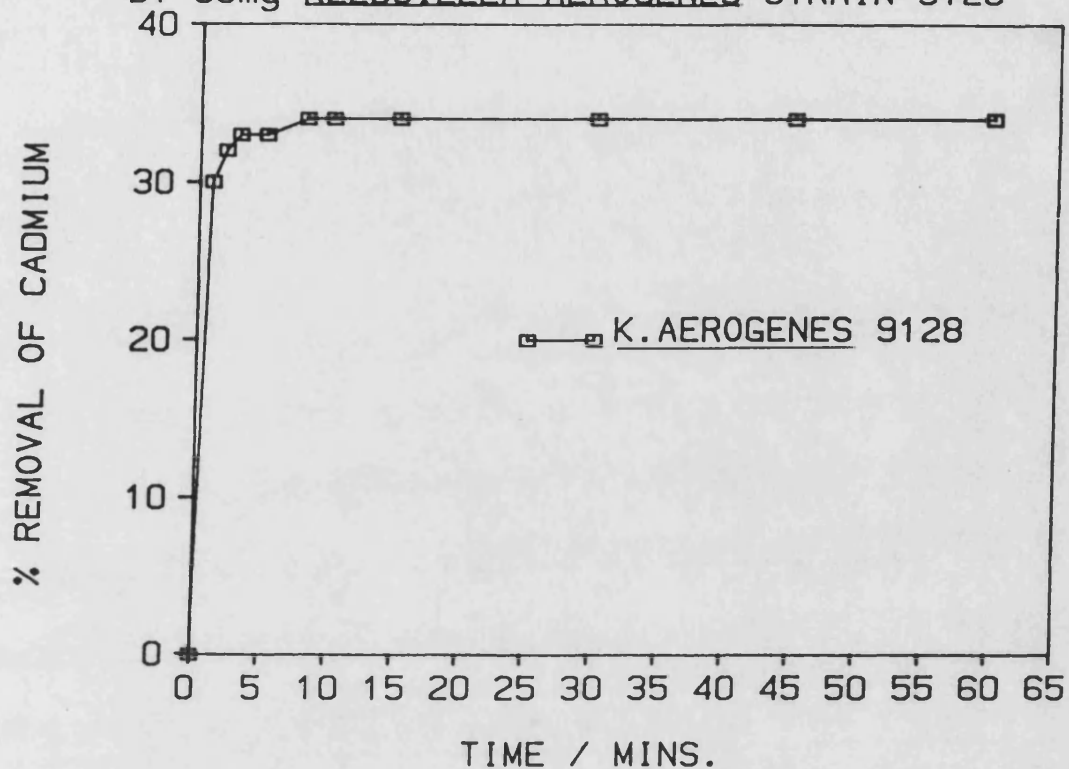
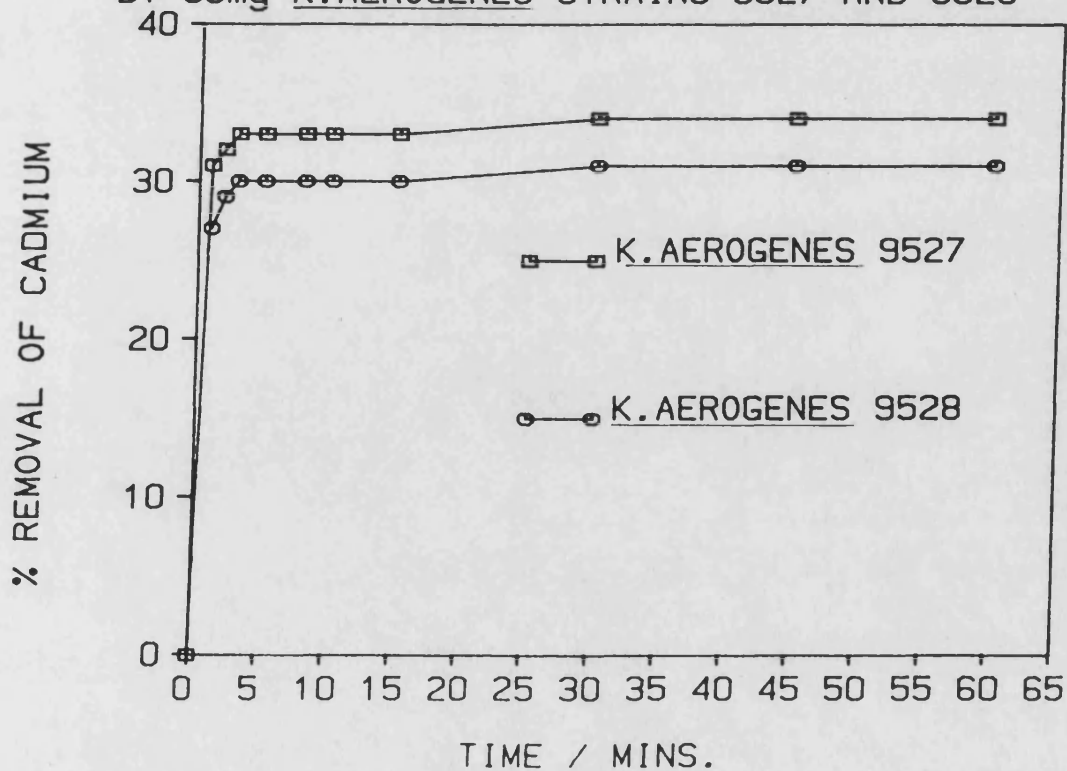


FIG.5B UPTAKE KINETICS FOR 10mg/L CADMIUM SOLUTION
BY 30mg K.AEROGENES STRAINS 9527 AND 9528



minutes and full equilibrium attained after 30 minutes. For these kinetic studies a different centrifugation regime was adopted to that used in the non-kinetic studies (see Materials and Methods). This rapid uptake suggests some form of adsorption of cadmium was occurring. The rapid uptake exhibited by these bacterial species is a useful trait. In any treatment system rapid accumulation of metal ions by biosorbent would minimise retention time allowing greater volumes of waste-water to be treated.

Equilibrium was fully attained by 30 minutes. These kinetic studies were used to determine a standard sampling time for all subsequent non-kinetic investigations. The project was based on the premise that biosorbent types (i.e. bacterial species) were to be characterised with regard to potential use in a waste-water treatment system. Thus although full equilibrium was normally achieved within 30 minutes, standard sampling time was selected as five minutes (by which time 90% of uptake was attained anyway) to represent a lower retention time in a full-scale process.

Rapid metal uptake has been observed by Horikoshi et al (1981), Strandberg et al (1981) and Friis and Myers-Keith (1986). Far slower rates of uptake have also been

observed (Shumate and Strandberg, 1985), but have been associated with metabolism dependent, intracellular metal binding (Gadd, 1986). Intracellular uptake of metal is not necessarily slow or metabolically linked though; Strandberg et al (1981) found that uranium rapidly accumulated by P.aeruginosa was bound within the cell. Hence the rapid accumulation of cadmium by the bacterial species used here provides no firm indication of sites of cadmium uptake.

Rapid accumulation of cadmium may be associated with passive physicochemical processes such as ion exchange or adsorption (Shumate and Strandberg, 1985); but no firm conclusions may be drawn in this respect until adsorption isotherms are analysed.

The rapidity of cadmium accumulation observed precluded any attempt to determine reaction order. In all cases only the final phase of the reaction was observed with the sampling technique employed.

3.1.2. Effect of initial cadmium concentration on biosorption

The uptake of cadmium by each bacterial species is represented here in two forms:

(1) the proportion (percentage) of cadmium removed from solution

(ii) the change in the specific binding capacity for cadmium of the biomass with increasing cadmium concentration. Specific binding capacity is designated 'Q' and expressed as mg cadmium bound per gramme of biomass.

Both expressions of the data satisfy particular requirements. From the percentage of cadmium removed from solution by a particular biomass concentration at a range of cadmium concentrations, conclusions may be drawn as to the relative efficiency of the biosorbent at different cadmium concentrations. Plotting specific cadmium uptake against a range of cadmium concentrations provides adsorption isotherms from which the characteristics of the biomass as a biosorbent may be assessed, and application of adsorption models may lead to some indication of the mechanism of cadmium uptake.

When determining the biosorptive capacity of biomass the amount of cadmium present is better defined as the amount in milligrammes available initially to the biomass as opposed to a concentration term (e.g. mg/L). This reduces ambiguity (often encountered in the available literature on metal biosorption) which can be associated with the concentration term (mg/L) as use of a small or large volume obviously alters the amount of cadmium available.

Stating the amount of cadmium present initially in mg gives a precise definition of the amount of cadmium available. Throughout the section the equivalent concentration (mg/L) is also provided in parentheses.

Figures 6-8 a show that proportional cadmium uptake decreases with increasing cadmium concentration. This is to be expected if it is assumed that the biomass only has a certain number of cadmium binding sites in or on it, which would become progressively occupied as the initial cadmium concentration increases. However, although more sites become filled as cadmium concentration increases (i.e. more metal is bound; figure 8b) the proportion of cadmium removed would be less (Table 1). Figures 5-8 indicate that differences exist in the cadmium uptake characteristics of the bacterial species. In terms of proportion of cadmium bound A.viscosus is more efficient than the other bacterial strains between 0.05 and 1.0mg(0.5-10mg/l). Beyond 1.0mg cadmium K.aerogenes strains 9128 and 9527 are most effective with K.aerogenes strain 9528 less so, but more efficient than A.viscosus and also the non-polysaccharide producing strains.

Figure 8b provides the adsorption isotherms for each bacterial species from 0.05 to 5.0mg (0.5-50mg/l) initial

FIG.6 % UPTAKE OF CADMIUM BY 30mg A.VISCOSUS AND
A.GLOBIFORMIS FROM 0.5 TO 50 mg/L INITIAL CADMIUM

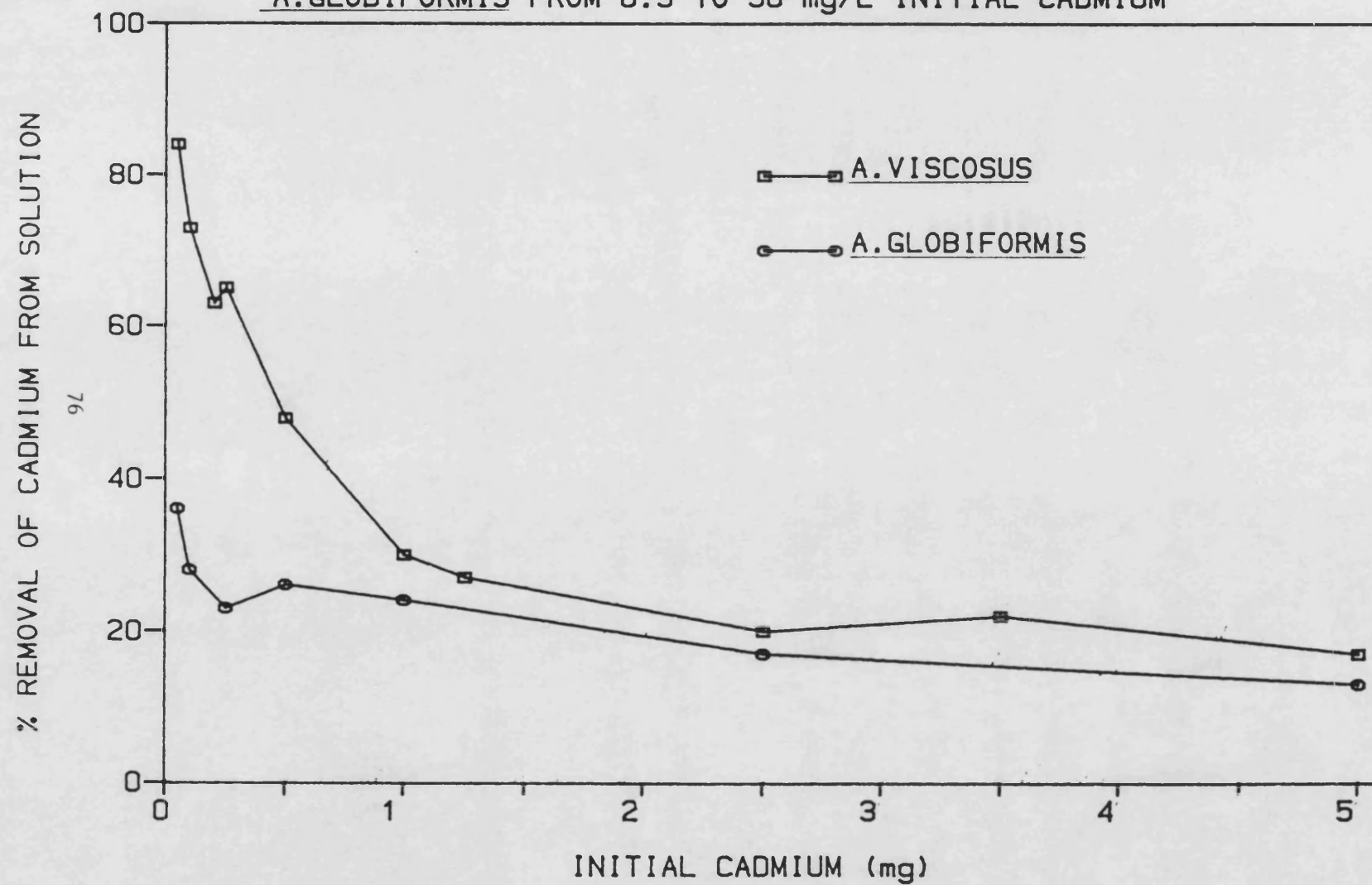


FIG.7 % UPTAKE OF CADMIUM BY 30mg PS.PUTIDA AND PS.CRUCIVIAE FROM 0.5 TO 50 mg/L INITIAL CADMIUM

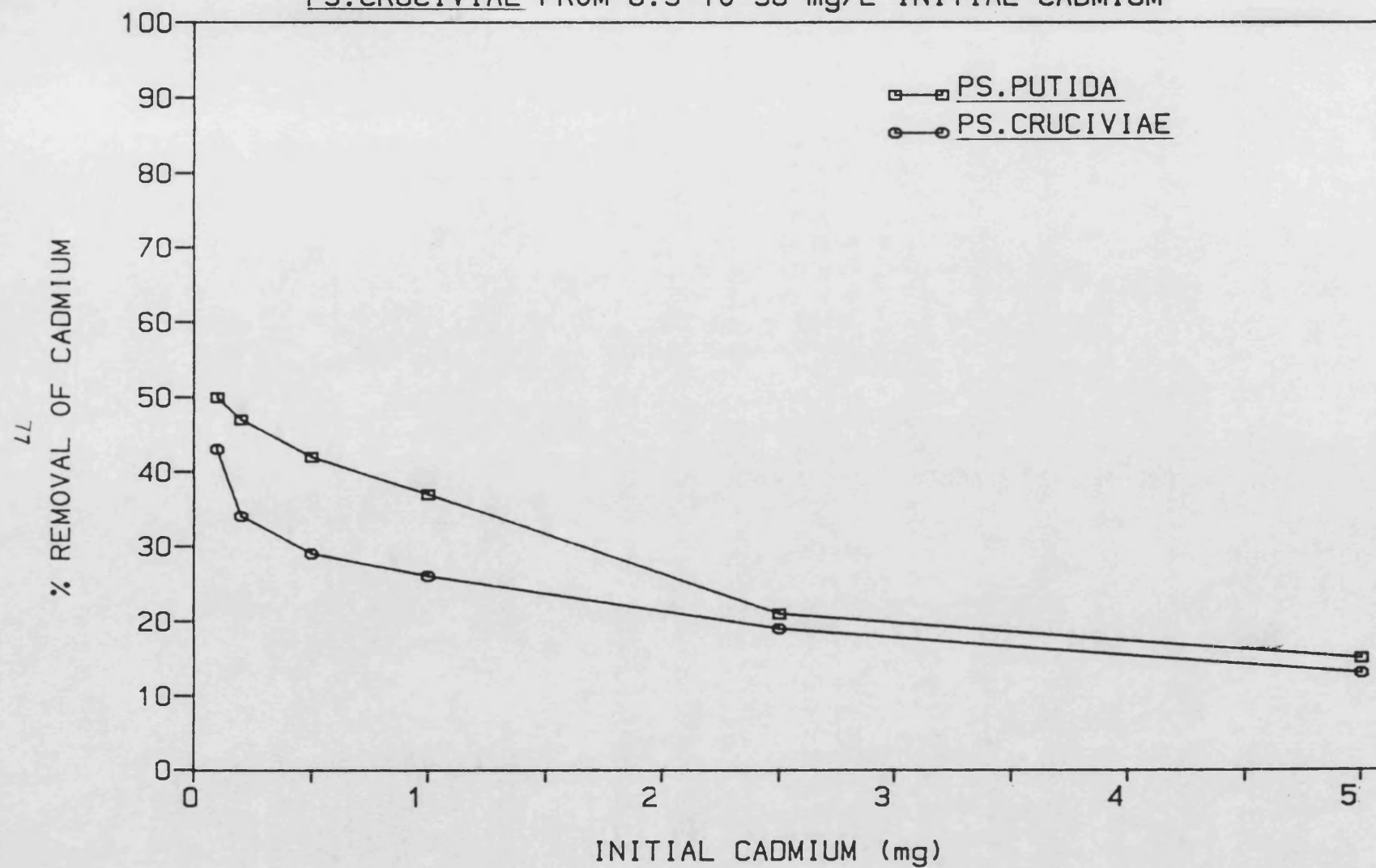


FIG.8A % UPTAKE OF CADMIUM BY 30 mg K.AEROGENES
STRAINS 9128 9527 AND 9528 FROM 2-50mg/L CADMIUM.

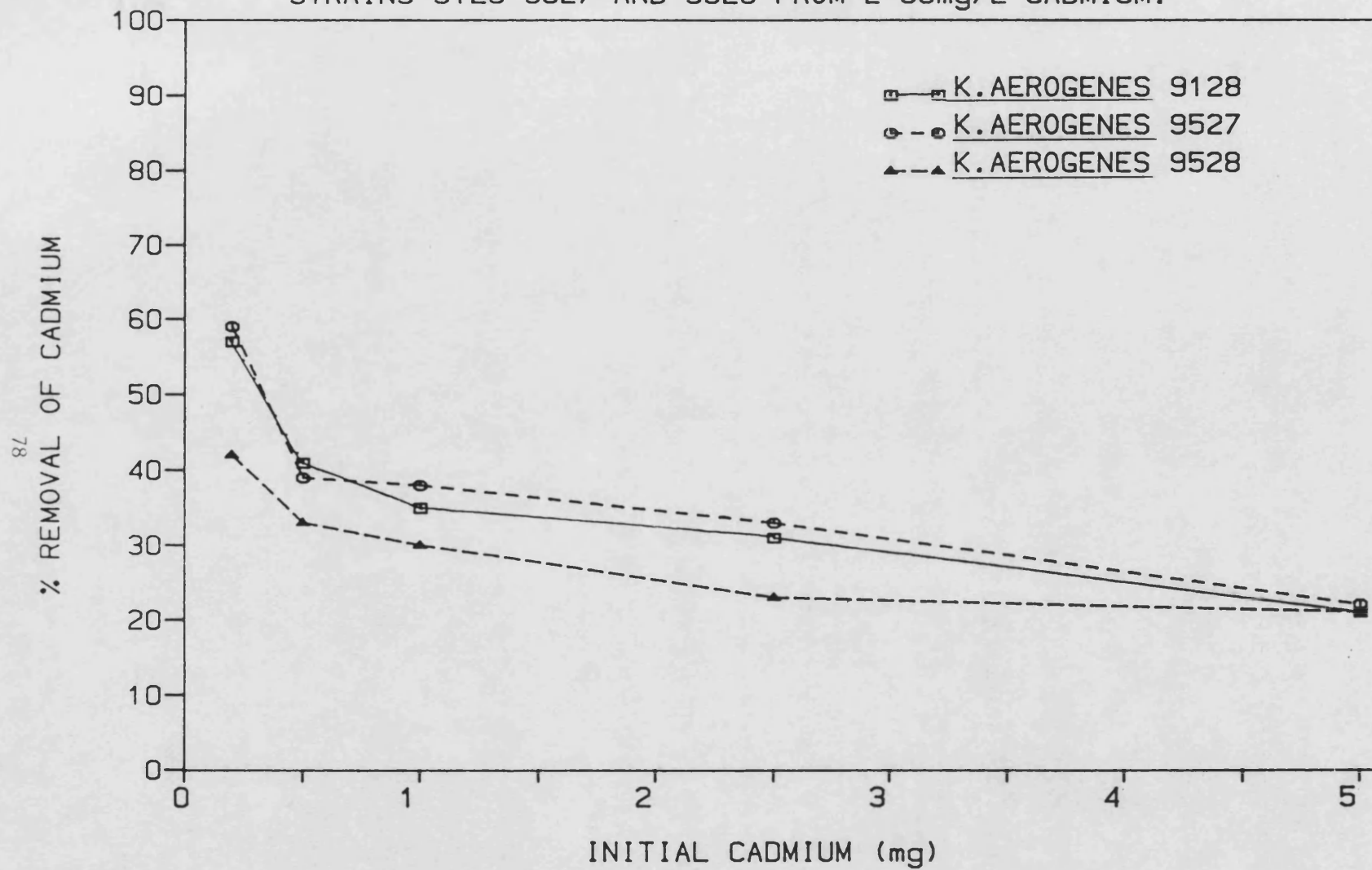


TABLE 1: % Removal of Cadmium by 30mg biomass

ORGANISM	CADMIUM CONCENTRATION (mg/l or ppm)											
	0.5	1.0	2.0	2.5	5.0	10.0	25.0	50.0	100.0	200.0	500.0	1000.0
<u>A.viscosus</u>	84	73	63	65	48	30	20	17	9	5	4	4
<u>A.globiformis</u>	36	28	ND	23	26	24	17	13	9	4	2	0
<u>P.putida</u>	ND	50	47	ND	42	37	21	15	6	ND		
<u>P.cruciviae</u>	ND	43	34	ND	29	26	19	13	8	ND		
<u>K.aerogenes</u> 9128	ND	ND	57	ND	41	35	31	21	12	8		
<u>K.aerogenes</u> 9527	ND	ND	59	ND	39	38	33	22	12	8		
<u>K.aerogenes</u> 9528	ND	ND	42	ND	33	30	23	21	9	5		

NB: ND = not determined

Temperature 20 C and pH 7±0.5

30mg biomass added to 100ml cadmium solution. Exposure time 5 minutes

that the bacterial species are favourable for use as an absorbent.

Over this cadmium concentration range, K.aerogenes strains 9527 , 912 8 and 9528 appear the best biosorbents in that order. A. viscosus proved next most efficient, followed by P. putida, P. cruciviae and finally A. globiformis in that order (figure 8b; table 2).

Apart from percentage removal of cadmium from solution the distribution of cadmium between biosorbent and solution may also be employed as an indicator of efficiency of a biosorbent at any particular concentration. This data (table 3) shows again that A.viscosus is most effective of the biosorbents at low concentration. This advantage for A.viscosus becomes lost beyond 0.5mg initial cadmium according to the distribution data. From Table 3 it is apparent that K.aerogenes biomass becomes the more effective biosorbent at 2.5mg (25mg/l) initial cadmium (particularly strains 9128 and 9527), but between 10.0 and 20.0mg (100-200mg/l) initial cadmium , the greater efficiency associated with these types decreases.

Returning to the adsorption isotherms of figure 5, the adsorption isotherms were rectilinear and of the isotherms described by Giles et al (1960) most closely

TABLE 2: Metal Uptake by Biomass (ratio of cadmium bound (mg) per gramme biomass)

ORGANISM	CADMIUM CONCENTRATION (mg/l or ppm)											
	0.5	1.0	2.0	2.5	5.0	10.0	25.0	50.0	100.0	200.0	500.0	1000.0
<u>A.viscosus</u>	1.4	2.4	4.2	5.4	8.0	10.0	16.7	28.3	30.0	33.3	66.7	133.3
<u>A.globiformis</u>	0.6	0.9	ND	1.9	4.3	8.0	14.2	21.7	30.0	26.7	33.0	0
<u>P.putida</u>	ND(a)	1.7	3.5	ND	7.0	12.3	17.5	25.0	20.0	ND		
<u>P.cruciviae</u>	ND	1.4	2.3	ND	4.8	8.7	15.8	21.7	26.7	ND		
<u>K.aerogenes</u> 9128	ND	ND	3.8	ND	6.8	11.7	25.8	35.0	40.0	53.3		
<u>K.aerogenes</u> 9527	ND	ND	3.9	ND	6.5	12.7	27.5	36.7	40.0	53.3		
<u>K.aerogenes</u> 9528	ND	ND	3.9	ND	5.5	10.0	19.1	35.0	30.0	33.0		

NB: ND(a) = not determined

Temperature 20 C and pH 7±0.5

30mg biomass added to 100ml cadmium solution. Exposure time 5 minutes

TABLE 3: Distribution of cadmium between biosorbent and solution (mg cadmium bound/mg residual cadmium)

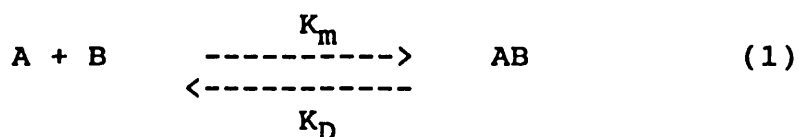
ORGANISM	CADMIUM CONCENTRATION/mg dm ⁻³											
	0.5	1.0	2.0	2.5	5.0	10.0	25.0	50.0	100.0	200.0	500.0	1000.0
<u>A.viscosus</u>	5.25	2.70	1.70	1.87	0.92	0.43	0.25	0.21	0.10	0.05	0.04	0.04
<u>A.globiformis</u>	0.56	0.39	ND	0.35	0.32	0.21	0.15	0.10	0.04	0.02	0.02	0.00
<u>P.putida</u>	ND	1.00	0.89	ND	0.72	0.59	0.27	0.18	0.06	ND		
<u>P.cruciviae</u>	ND	0.75	0.52	ND	0.41	0.35	0.24	0.15	0.09	ND		
<u>K.aerogenes</u> 9128	ND	ND	1.33	ND	0.70	0.54	0.45	0.27	0.14	0.05	ND	
<u>K.aerogenes</u> 9527	ND	ND	1.44	ND	0.64	0.61	0.49	0.28	0.14	0.09		
<u>K.aerogenes</u> 9528	ND	ND	0.72	ND	0.49	0.43	0.30	0.27	0.10	0.05		

NB: ND = not determined

Temperature 20 C and pH 7±0.5

30mg biomass added to 100ml cadmium solution.

fit the L-type isotherm (Langmuir isotherm). The Langmuir isotherm may be represented as follows. Where a solute 'A' (in this case cadmium) is adsorbed or complexed with a ligand 'B', the reaction may be represented as:



The binding capacity of an absorbent may be related to this reaction as:

$$\text{Specific binding capacity 'Q'} = \frac{Q_m K_m C}{1 + K_m C} \quad (2)$$

$$\text{giving} \quad Q = \frac{Q_m C}{K_D + C} \quad (3)$$

Equation (3) may be linearised to give

$$\frac{C}{Q} = \frac{K_D}{Q_m} + \frac{C}{Q_m} \quad (4)$$

where C = residual cadmium concentration
 Q = specific binding capacity
 Q_m = binding capacity at equilibrium
 K_m = association constant
 K_D = dissociation constant.

Thus a plot of residual cadmium concentration C (mg/l) against binding capacity ' Q ' (mg cadmium per gramme biomass) will give a straight line of slope $1/Q_m$ if the data fits a Langmuir isotherm. Langmuir isotherms have already been observed in use of some microorganisms and their products (Sterritt and Lester, 1986).

Another isotherm that has been applied to metal adsorption by biomass is the Freundlich isotherm (Sterritt and Lester, 1986); particularly to fungi (Tsezos and Volesky, 1981; Kiff and Little, 1986; Gadd, 1986). The Freundlich equation:

$$Q = K C^{1/n} \quad (5)$$

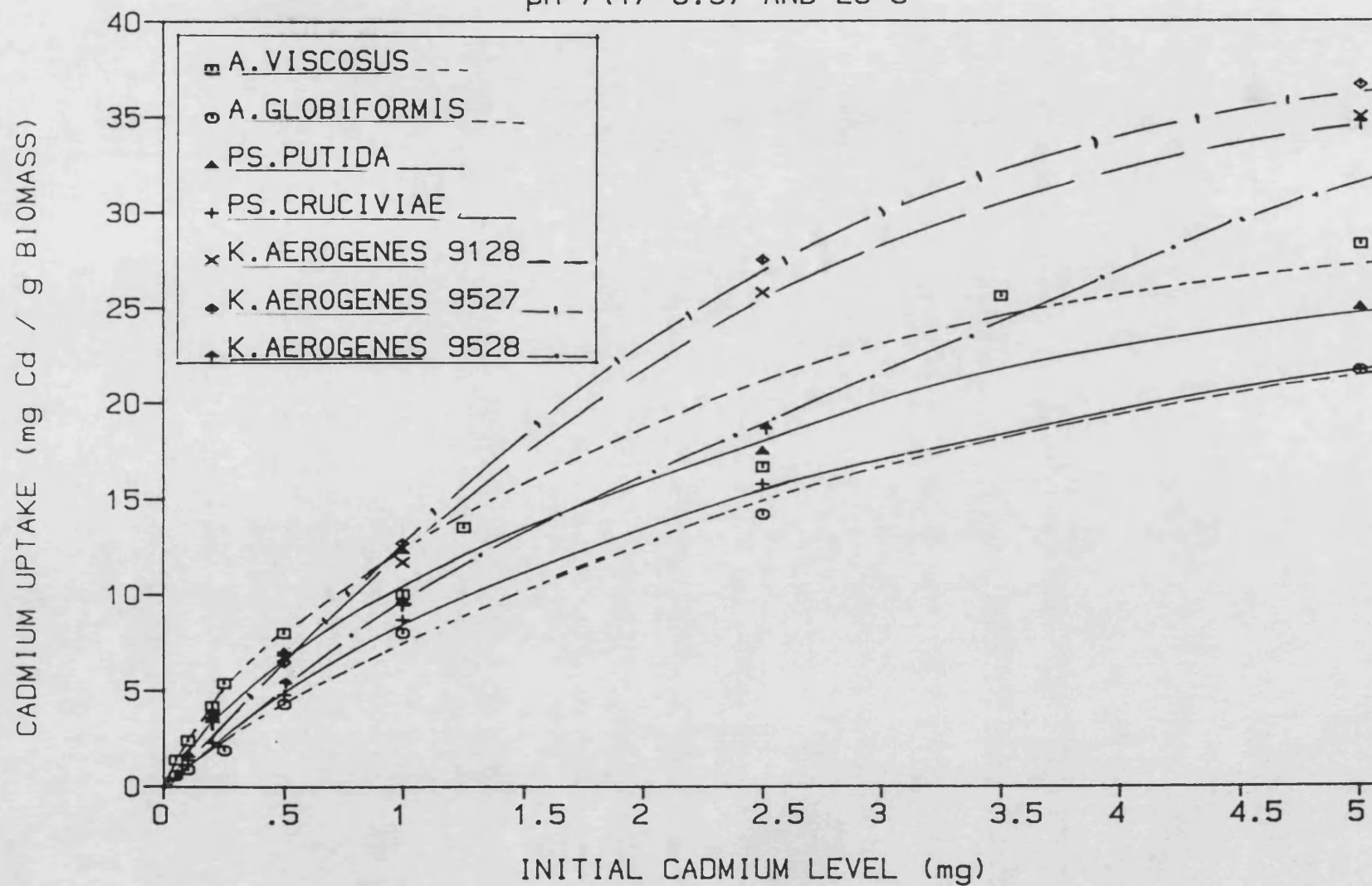
where Q is binding capacity, C equilibrium solute concentration and K and n are constants, may be linearised as

$$\ln Q = \ln K + (1/n) \ln C \quad (6)$$

A plot of $\ln Q$ against $\ln C$ would be linear if data fitted a Freundlich isotherm with $1/n$ as slope and K as intercept (Tsezos and Volesky, 1981).

For each bacterial species cadmium biosorption data within the range 0.05-10.0 mg(0.5-100 mg/l) initial

FIG.8(B) CADMIUM ADSORPTION BY 30mg BIOMASS AT
pH 7(+/-0.5) AND 20°C



cadmium concentration was linearised for fitting to both Langmuir and Freundlich isotherms and the goodness of fit assessed by calculating the correlation coefficient.

In addition, the procedure was repeated in the 0.05 - 20.0mg (0.5-200mg/l) range for A.viscosus, A.globiformis and all three K.aerogenes strains and in the 0.05-100.0mg (0.5-1000mg /l) range for both Arthrobacter species. The results of this investigation are presented in table 4.

For all the bacterial species except A.globiformis the Langmuir isotherm provided a better fit for the data than the Freundlich isotherm. However, it should be noted that in several cases the difference in correlation between the two types of isotherm was minor (e.g. P.cruciviae in the initial cadmium range 0.05-10.0mg (0.05-100mg/l); A.globiformis in the range 0.05-10.0mg (0.5-100mg/l) (and 0.05-20.0mg, etc.).

This has also been observed with two fungi (deRome and Gadd, 1987). In most cases the correlation was significant (i.e. >95%), but it is interesting to note that for both Arthrobacter species (the only species characterised up to 1000mg/l) the correlation of both models decreased beyond this level of significance between 20.0 and 100.0mg initial cadmium.

TABLE 4: Correlation of Linearised Biosorption Isotherms

BACTERIAL SPECIES

Initial Cadmium Concentration Range (mg/l)	<u>K.aerogenes</u> 9128		<u>K.aerogenes</u> 9527		<u>K.aerogenes</u> 9528		<u>A.viscosus</u>		<u>A.globiformis</u>		<u>P.putida</u>		<u>P.cruciviae</u>	
0.5 - 100	0.995	0.953	0.994	0.966	0.980	0.971	0.985	0.921	0.985	0.992	0.992	0.951	0.997	0.993
0.5 - 200	0.958	0.901	0.992	0.978	0.946	0.902	0.995	0.492	0.996	0.981	-	-	-	-
0.5 - 1000	-	-	-	-	-	-	0.828	0.576	0.431	0.770	-	-	-	-
Isotherm	LA	FR	LA	FR	LA	FR	LA	FR	LA	FR	LA	FR	LA	FR

Isotherms: LA = Langmuir; FR = Freundlich

While all other biomass types showed a slightly better fit for the Langmuir model, A.globiformis biosorption of cadmium fit the Freundlich model better between 0.05-10.0 and 0.05-100.0mg (i.e. 0.5-1000mg/l) . Thus, on balance,

A.globiformis biosorption would appear to fit the Freundlich isotherm better than the Langmuir for cadmium. In conclusion, cadmium biosorption appeared to give a significant fit to Langmuir adsorption, although in many cases both Langmuir and Freundlich isotherms could fit the data reasonably well. For A.globiformis the Freundlich isotherm provided the better fit for the data. For a Langmuir isotherm as the amount of solute bound increases, progressively less solute is bound because the probability of a solute molecule finding a binding site as sites become occupied decreases (Giles et al, 1960).

Of the bacterial strains employed A.viscosus was the most efficient biosorbent between 0.05 and 0.5mg(0.5-5mg/l) initial cadmium. Between 0.5 and 20.0mg/l cadmium K.aerogenes strains 9128, 9528 and 9527 were the most effective biosorbents. These three strains all produce extracellular polysaccharide, but their effectiveness cannot be directly attributed to capsular polysaccharide production on this data alone for one particular reason. K.aerogenes strain 9528 was a polysaccharide producer,

but non-capsule producing mutant selected for comparison with capsule-producing strains 9128 and 9527. Even though K.aerogenes 9528 did not produce a capsule and was not as efficient at cadmium biosorption as the two capsular strains until 5mg(50mg/l) initial cadmium, it still was more effective than the other bacterial species from 2.5mg initial cadmium onward. This might indicate that polysaccharide production was responsible for high uptake. However, Aiken et al (1982) found that K.aerogenes could be induced to produce a cadmium detoxification system by means of sulphide production and precipitation of cadmium as cadmium sulphide. This took far longer to induce however than the five minutes exposure to cadmium solution biosorbent underwent in the investigation, but unless proved otherwise, such a system might mediate cadmium removal from solution by K.aerogenes. This example illustrates that conclusions cannot be drawn as to the advantages, if any, of using exopolymer producing bacteria as a biosorbent without some knowledge of the mechanism and main sites of cadmium binding.

3.2. Environmental and other factors influencing cadmium uptake

3.2.1. Temperature

The effect of temperature over the range 5-40°C on the total amount of cadmium bound and on the kinetics of biosorption was investigated. Figures 9-13 are the isotherms for each of the bacterial species examined. For all species increase in temperature resulted in an increase in the amount of cadmium bound at equilibrium (taken as 30 minutes in this experiment) and also an increase in rate of uptake (Figures 9-13). The increase in cadmium bound with increase in temperature was linear (Figures 14 and 15) but not large. The enthalpy of cadmium adsorption was determined for each species from a plot of logarithms of the distribution coefficient (K_D) of cadmium in solution againsts reciprocal of temperature. These enthalpies of adsorption (Table 5) were all quite low. Nakajuma et al (1982) and Friis and Myers-Keith (1986) have also observed minor temperature effects on uranium biosorption by Chlorella (an algae) and Streptomyces respectively. Nakajuma et al also noted that their data indicated that uranium adsorption was endothermic, which was also observed with bacterial species employed here (Table 5.) .

FIG.9 EFFECT OF TEMPERATURE ON BIOSORPTION FROM A 1mg/L CADMIUM SOLUTION BY 30mg *A.VISCOSUS*

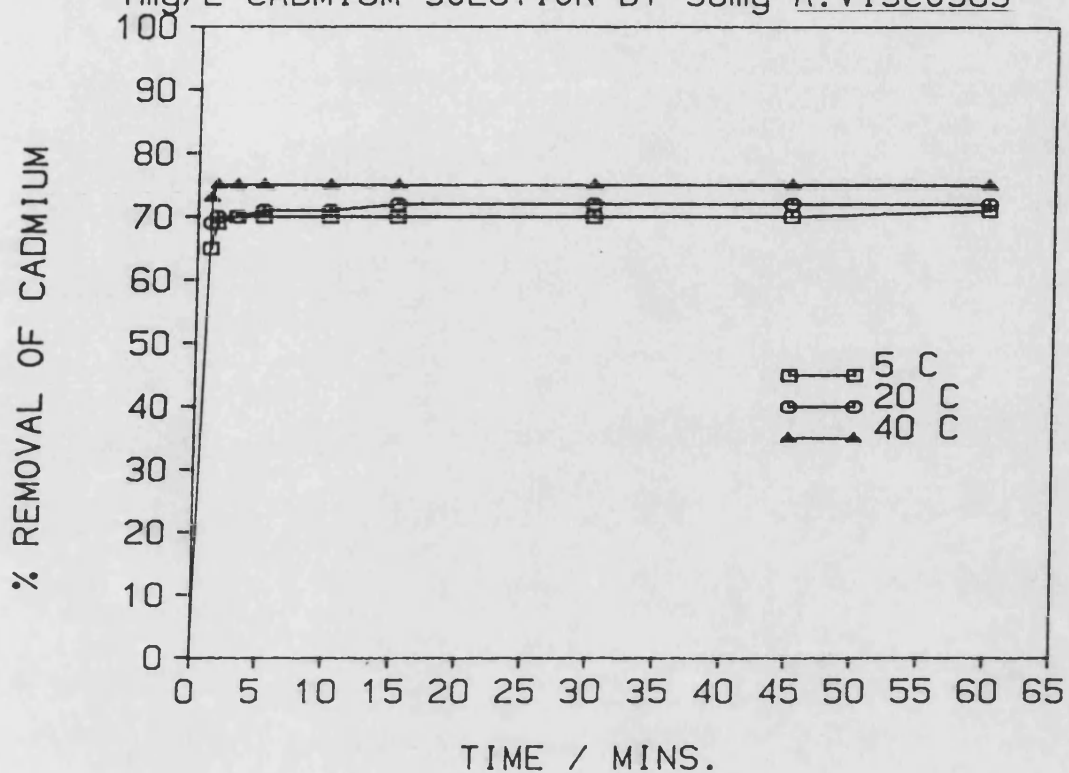


FIG.10 EFFECT OF TEMPERATURE ON BIOSORPTION FROM A 1mg/L CADMIUM SOLUTION BY 30mg *A.GLOBIFORMIS*

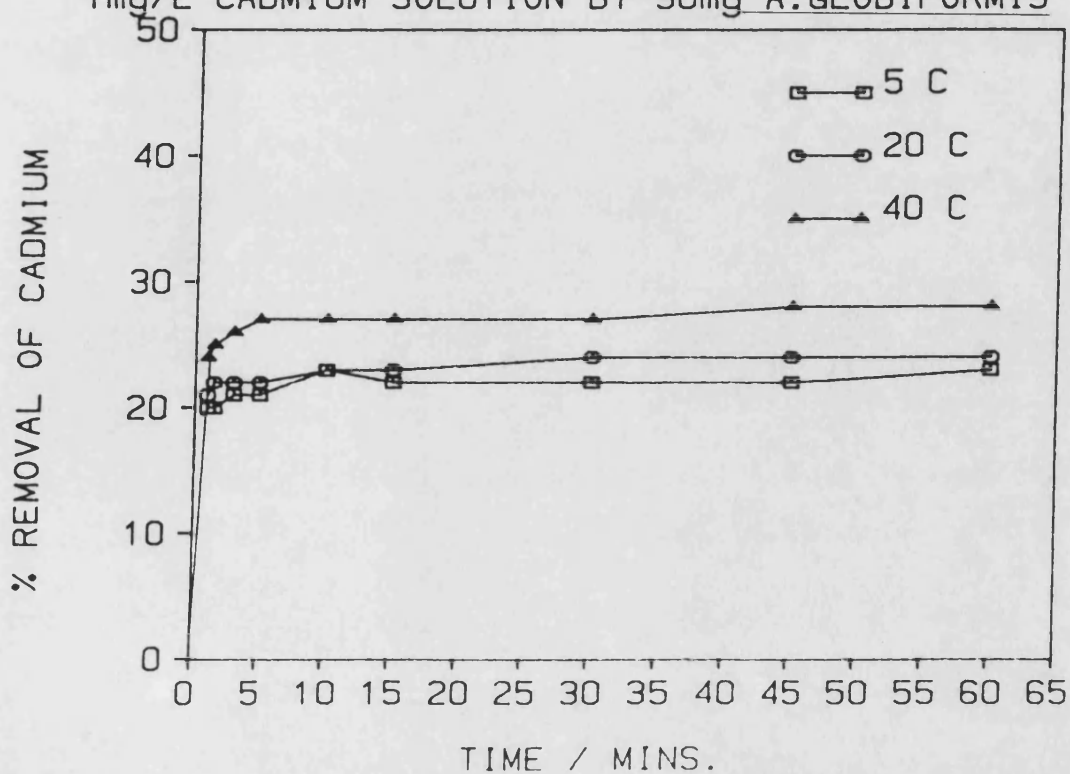


FIG.11 EFFECT OF TEMPERATURE ON BIOSORPTION FROM A 5mg/L CADMIUM SOLUTION BY 30mg PS.CRUCIVIAE

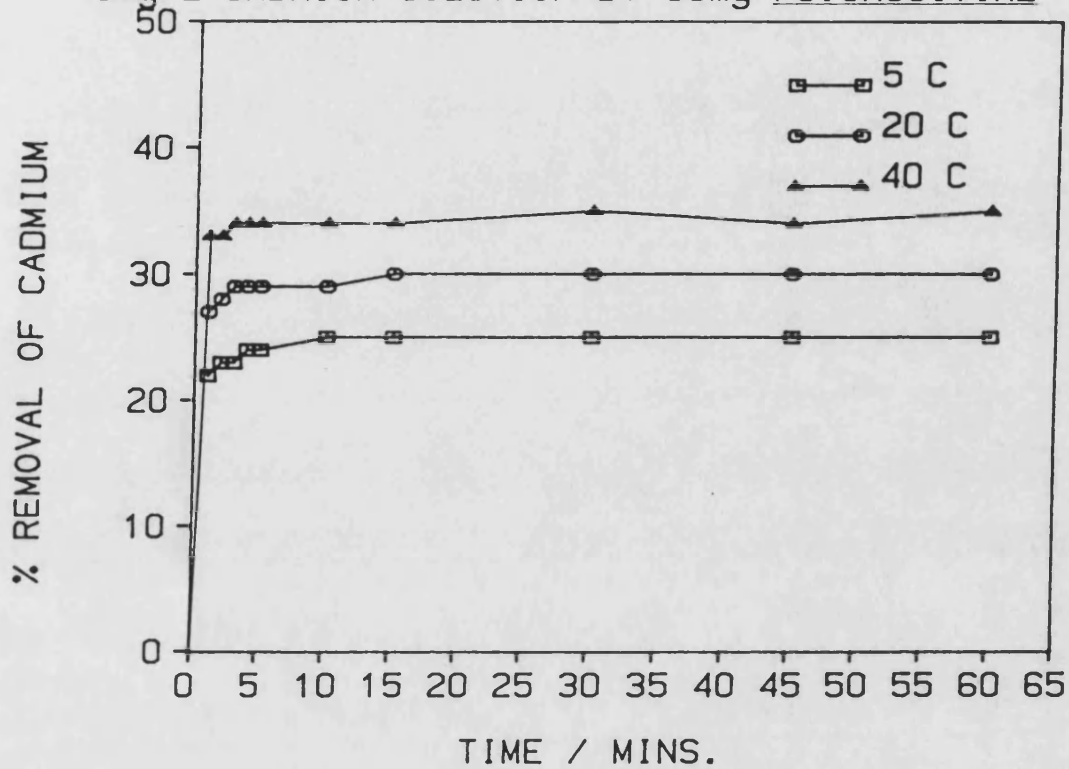


FIG.12 EFFECT OF TEMPERATURE ON BIOSORPTION FROM A 10mg/L CADMIUM SOLUTION BY K.AEROGENES 9128

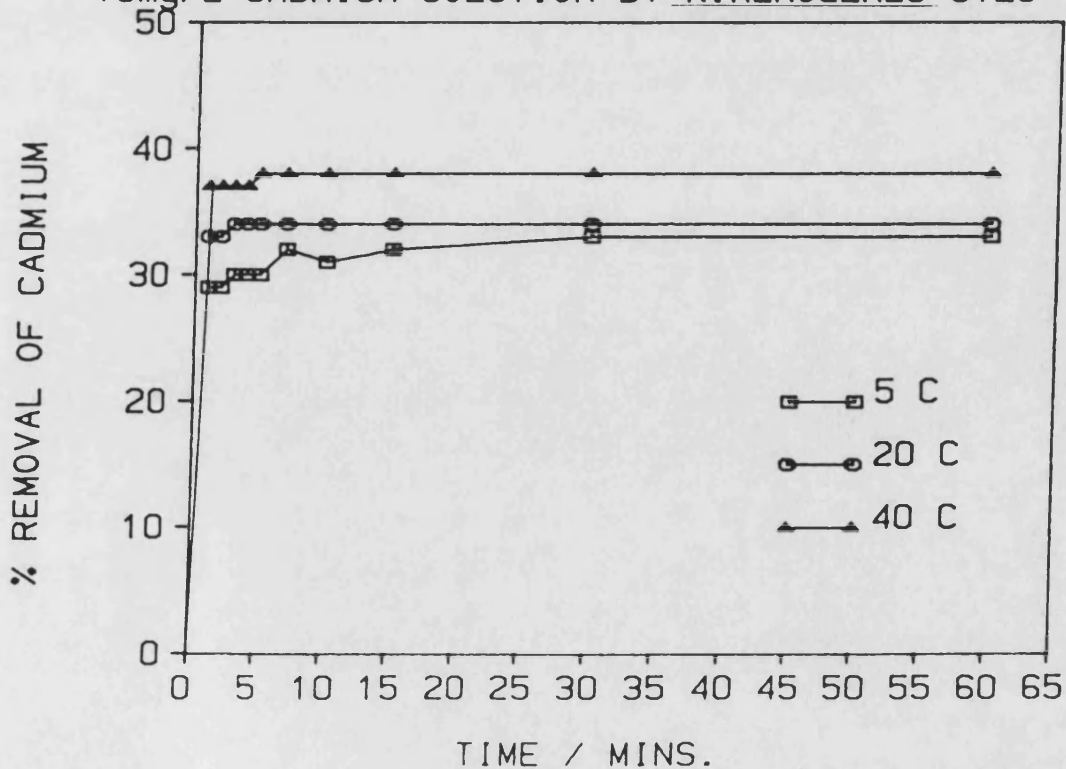


FIG.13 EFFECT OF TEMPERATURE ON BIOSORPTION FROM A
10mg/L CADMIUM SOLUTION BY 30mg K.AEROGENES 9128

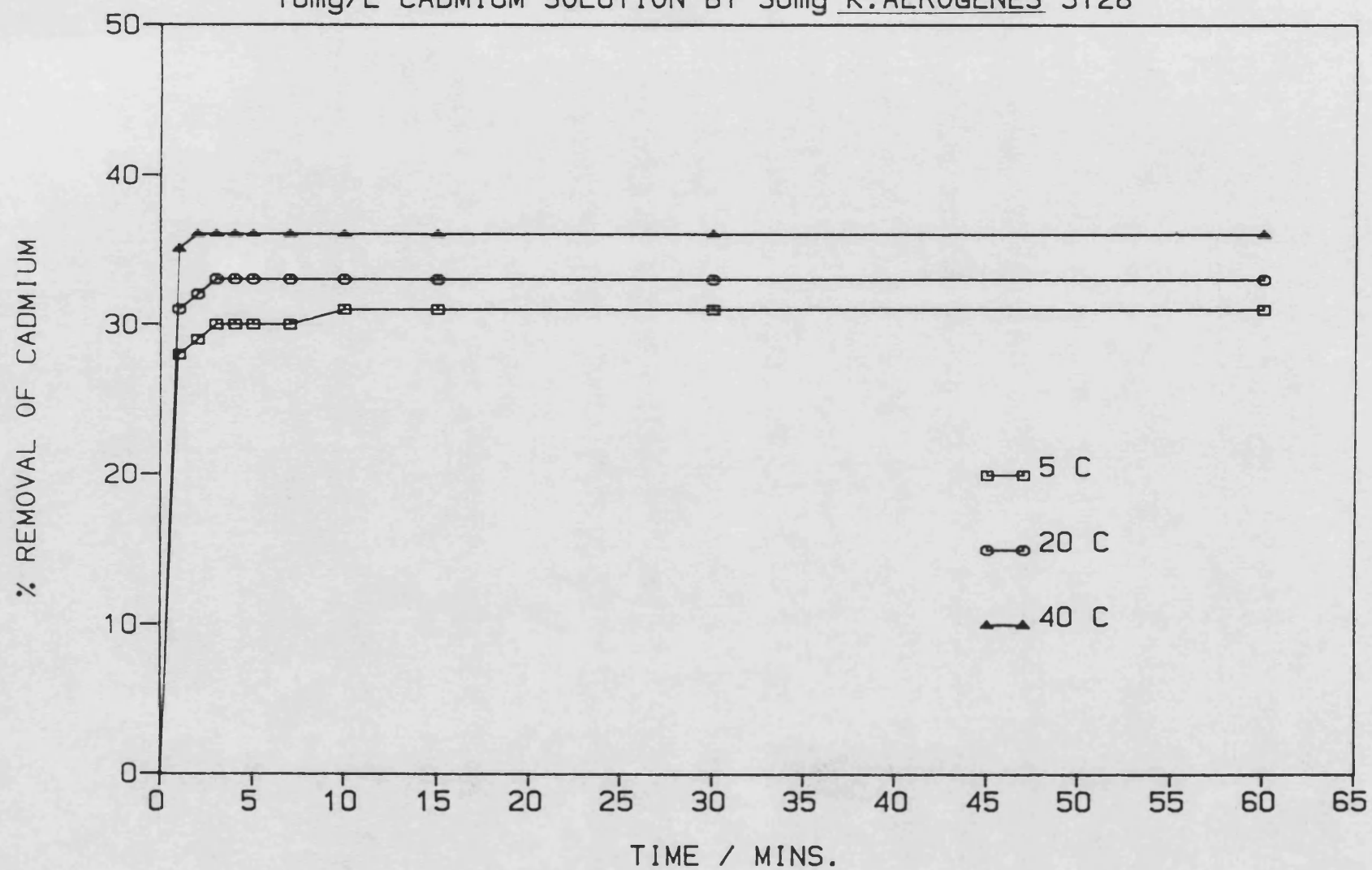


FIG.14 EFFECT OF TEMPERATURE ON CADMIUM BINDING AT EQUILIBRIUM BY PS.CRUCIVIAE AND K.A.9128 AND 9528

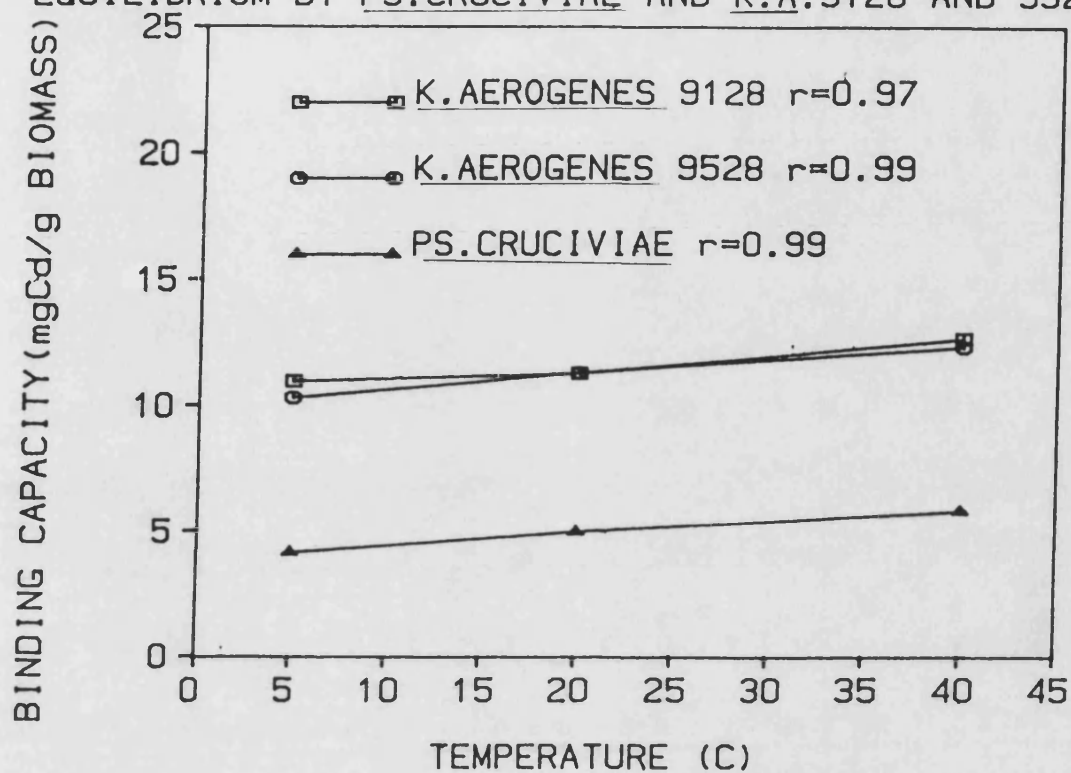


FIG.15 EFFECT OF TEMPERATURE ON CADMIUM BINDING AT EQUILIBRIUM BY A.VISCOSUS AND A.GLOBIFORMIS

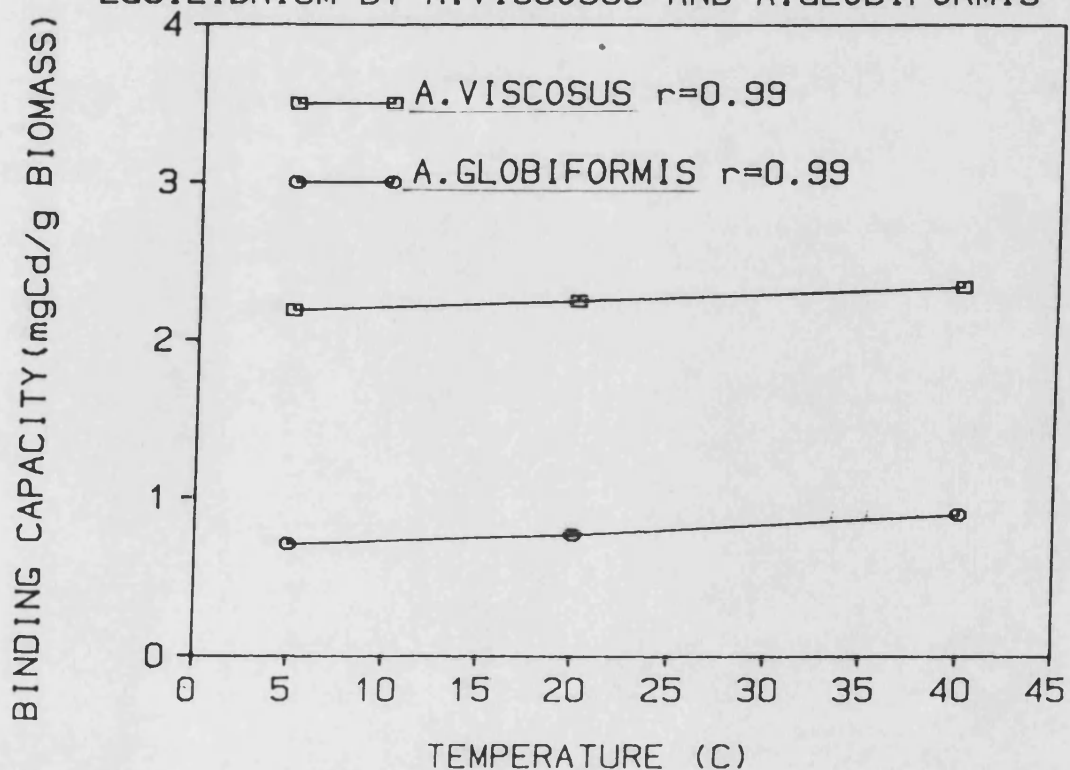


Table 5 Enthalpy of adsorption by biomass associated with increase in temperature from 5 to 40°C.

Organism	Enthalpy of adsorption (KJ/mol)	Cadmium conc(mg/l)
<u>P.cruciviae</u>	+9.6	5
<u>A.globiformis</u>	+5.8	1
<u>K.aerogenes</u> 9128	+6.1	10
<u>K.aerogenes</u> 9528	+4.4	10

3.2.2. Effect of pH on cadmium biosorption

The concentration of cadmium in solution decreases as pH increases. Above pH 9.5 in an ideal solution, the residual cadmium concentration does not exceed 1.12mg/l (Rauhut, 1978). Hence the effect of pH on biosorption was determined at a concentration of 1mg/l for A.viscosus, A.globiformis, P.cruciviae and K.aerogenes 9128, and also determined for 0.5mg/l cadmium for A.viscosus. Using a concentration of 1mg/l should theoretically allow the uptake of cadmium to be determined up to pH 9 without the cadmium concentration being reduced by precipitation of cadmium as cadmium hydroxide.

The general trend exhibited by the bacterial species was for cadmium biosorption to reach an optimum between pH 6 and 7 (e.g. for A.viscosus the optimum was determined to be pH 6.5). No cadmium biosorption was evident between pH 1 to 4; beyond pH 7 biosorptive capacity either remained approximately constant or decreased.

The decrease of cadmium removal from solution with increase in hydrogen ion concentration seemed to suggest a competition between hydrogen ions and cadmium ions for the available binding sites of the bacterial cells. Between the pH values of 1 and 4, cadmium is totally excluded from binding to the biomass. For A.viscosus at 1mg/l the decrease in binding of cadmium between uptake recorded at the lowest pH, 4.5, and the optimum pH for binding, 6.5, was approximately linear (Figure 19). A linear relationship between hydrogen ion concentration and metal ion displacement has been observed for two fungi (de Rome and Gadd, 1987) and an actinomycete (Friis and Myers-Keith, 1986). The correlation for this relationship was relatively low (0.83), but the slope of the regression line (1.91) suggests that two hydrogen ions are required to displace a cadmium ion from biomass. Further investigation is required to validate properly this relationship. What may be deduced from the data is that competition does exist between hydrogen ions and cadmium ions for binding sites. As the hydrogen ion

FIG.16A EFFECT OF pH ON UPTAKE FROM 1mg/L CADMIUM SOLUTION BY A.GLOBIFORMIS

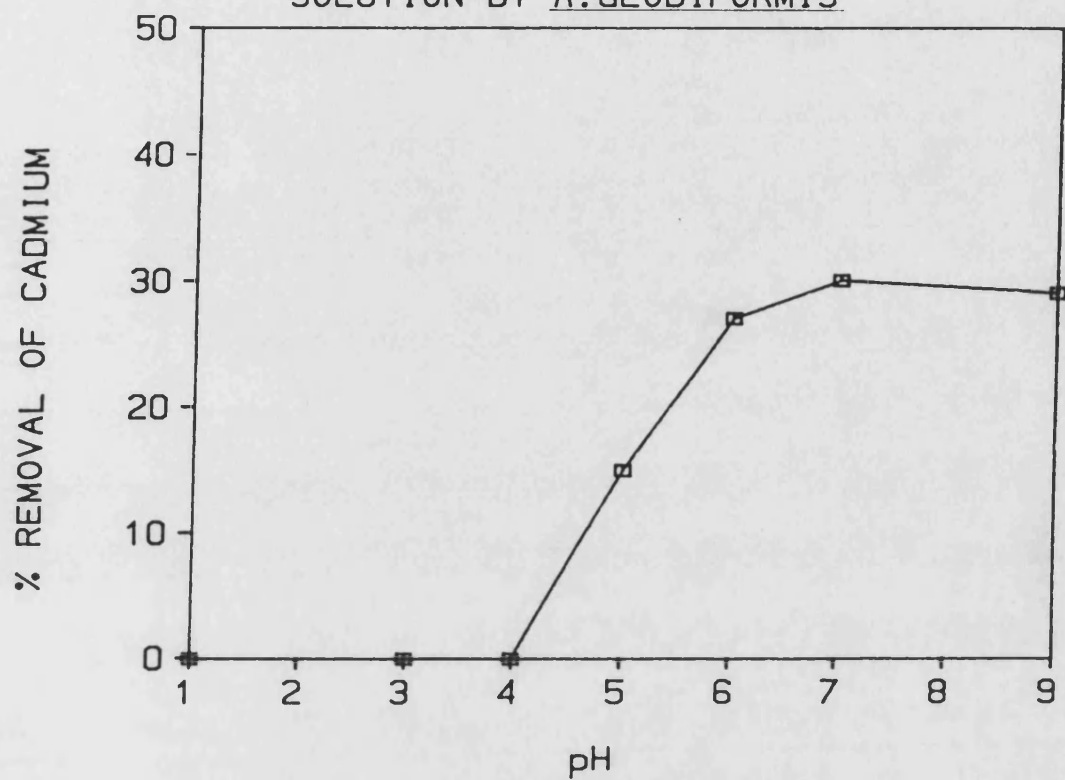


FIG.16B EFFECT OF pH ON UPTAKE FROM 0.5mg/L CADMIUM SOLUTION BY A.VISCOSUS

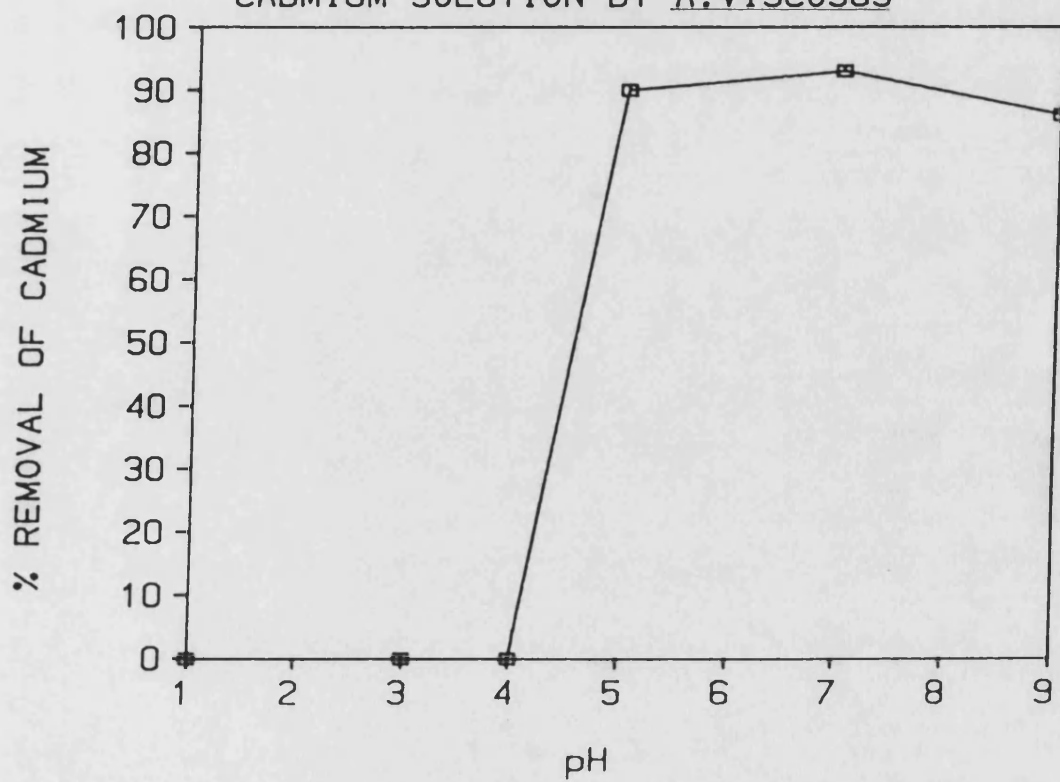


FIG.17A EFFECT OF pH ON UPTAKE FROM 1mg/L CADMIUM SOLUTION BY PS.CRUCIVIAE

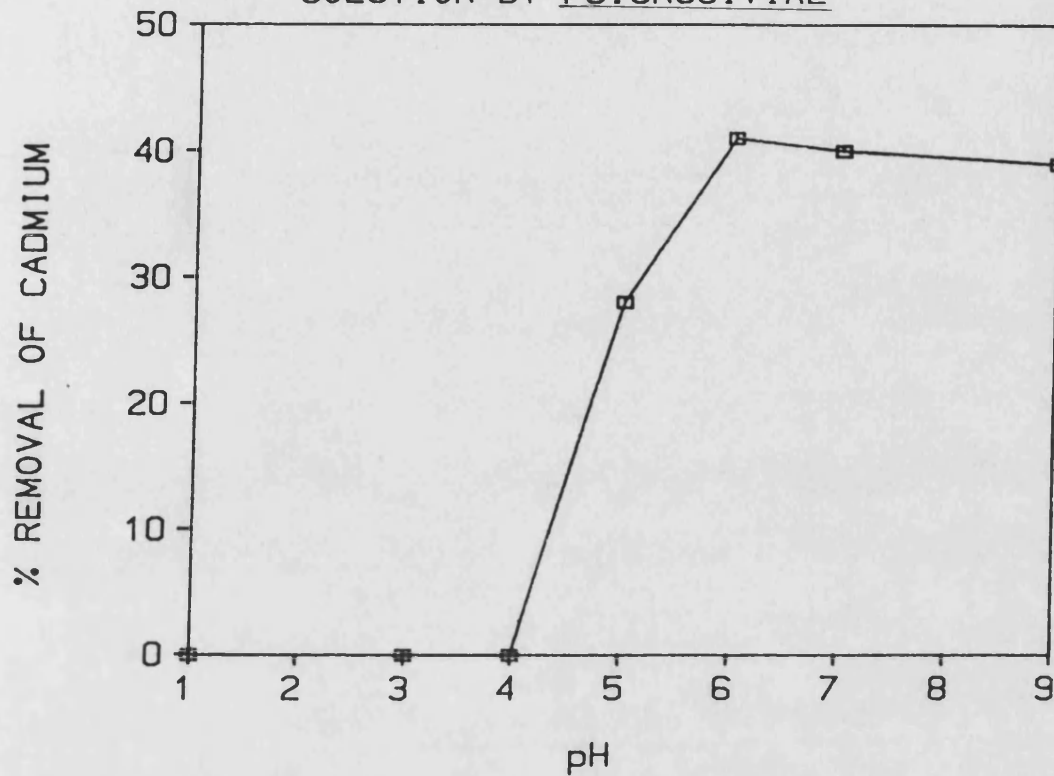


FIG.17B EFFECT OF pH ON UPTAKE FROM 1mg/L CADMIUM SOLUTION BY K. AEROGENES 9128

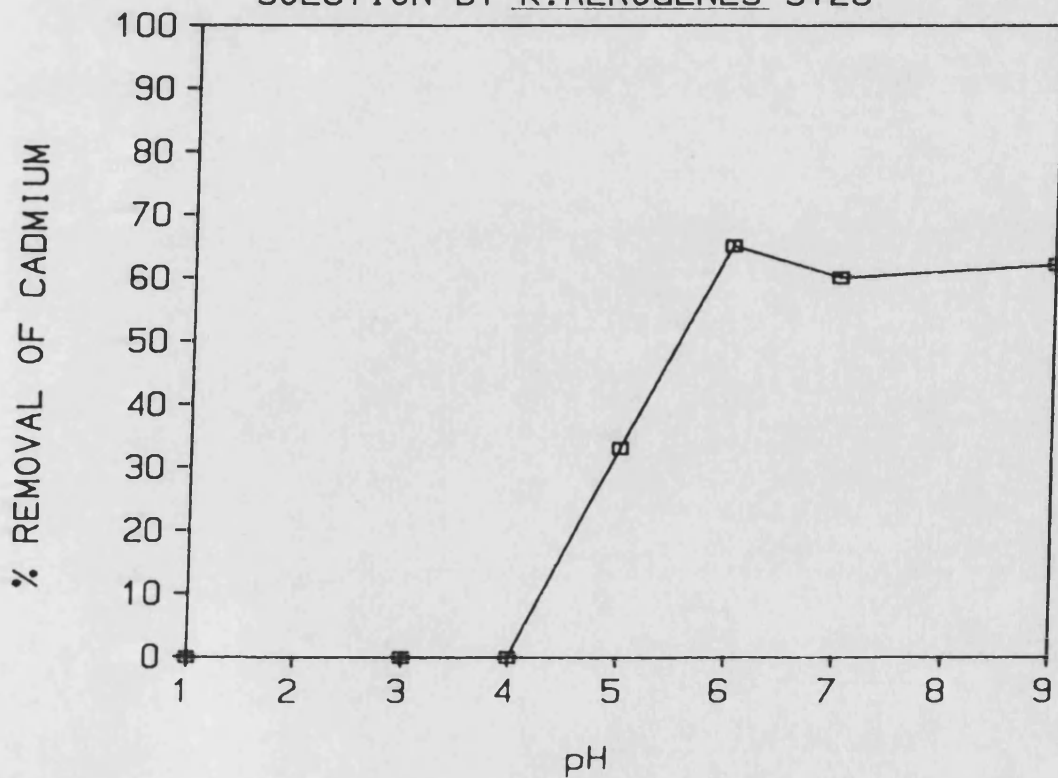


FIG.18 EFFECT OF pH ON UPTAKE FROM 1mg/L CADMIUM
BY A.VISCOSUS

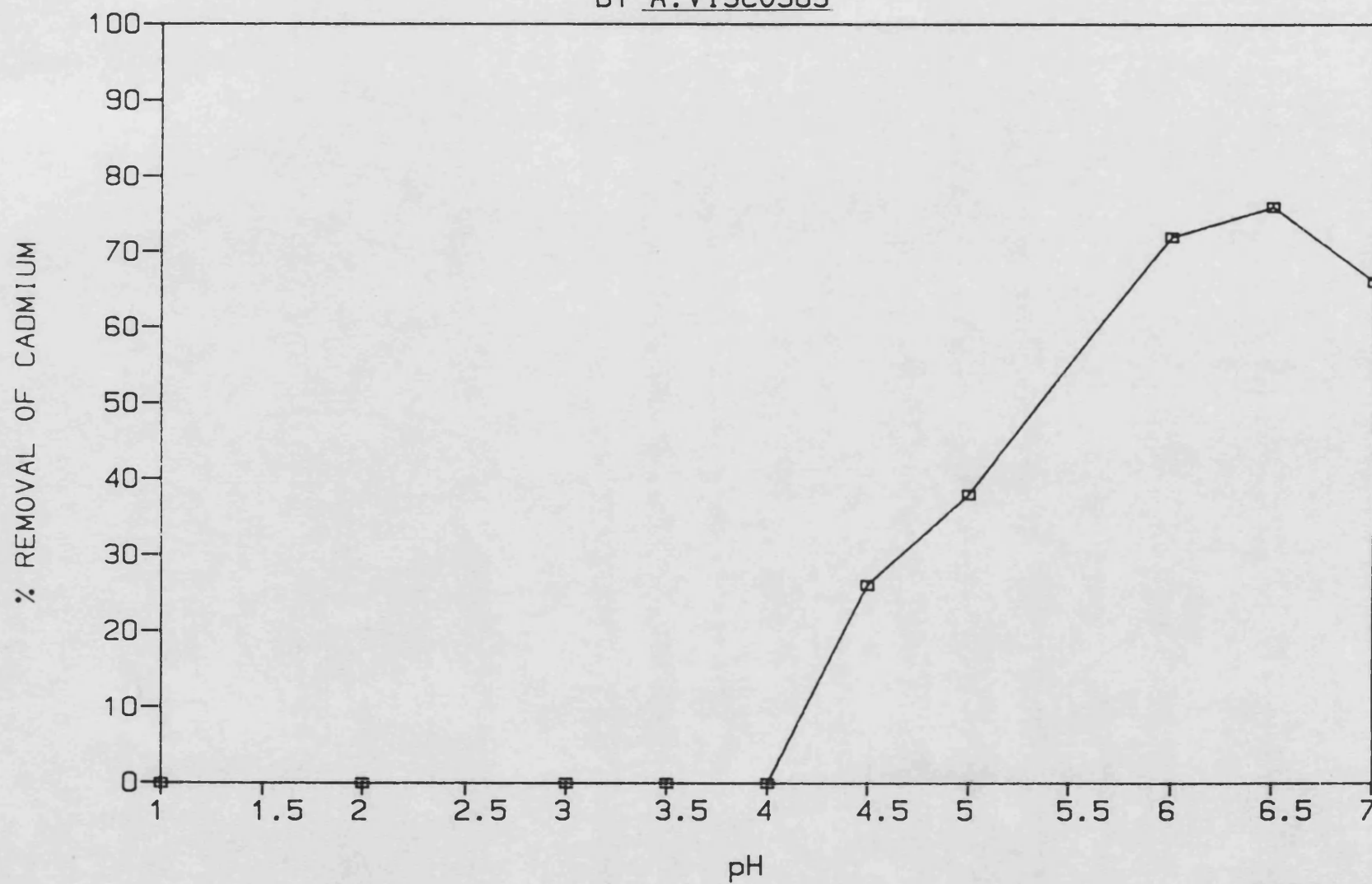
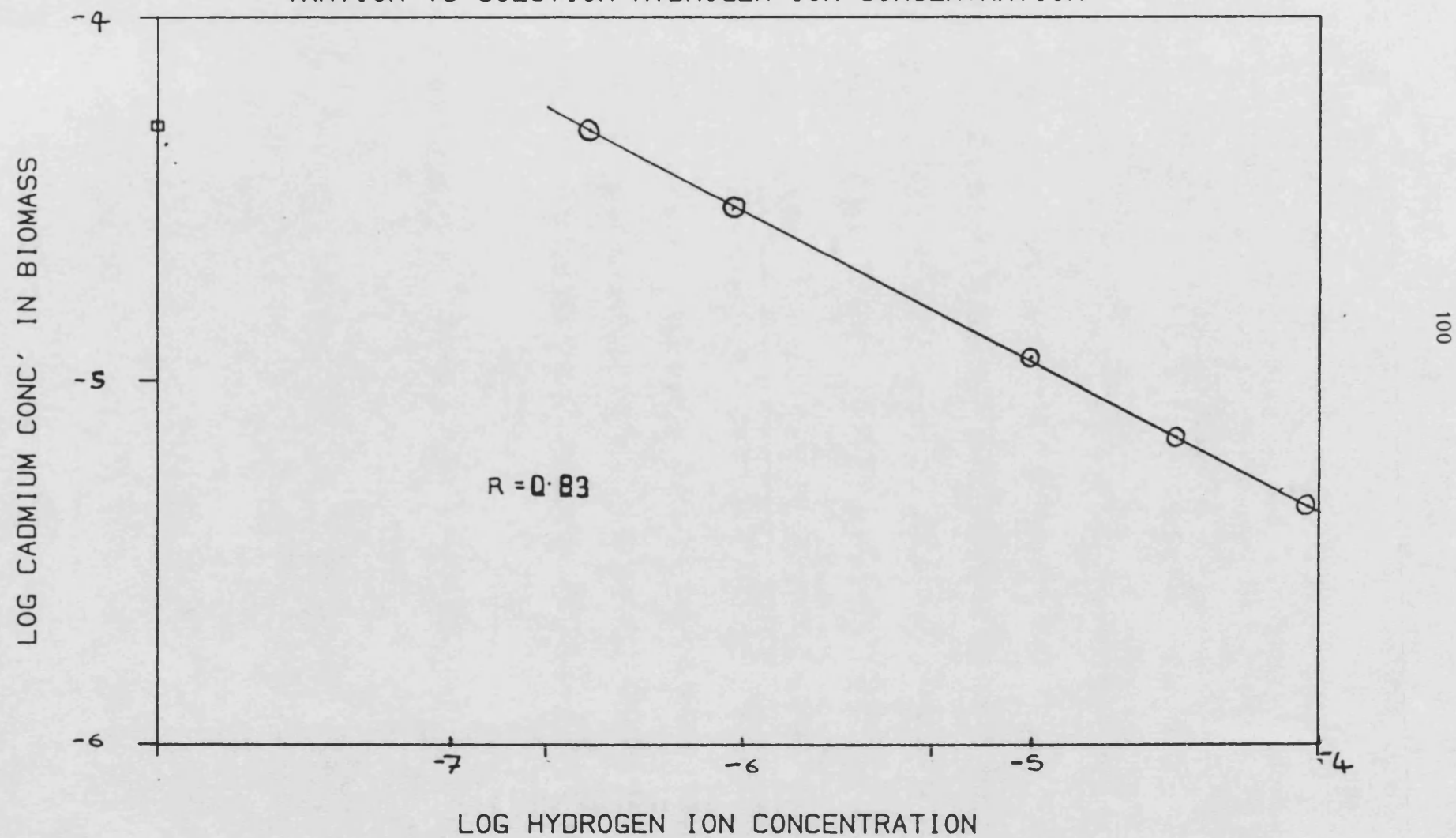


FIG.19 REGRESSION PLOT FOR BIOMASS CADMIUM CONCENTRATION vs SOLUTION HYDROGEN ION CONCENTRATION



concentration in solution increases, the concentration of cadmium ions associated with biomass decreases. This effect has been observed in cadmium uptake by Z. ramigera (Norberg and Persson, 1984) and metal accumulation by a number of biosorbents (Shumate and Strandberg, 1985) and such competition between hydrogen ions and cadmium ions would be responsible for Babich and Stotksy's observation that cadmium toxicity to some bacteria is increased at alkaline pH. Competition between hydrogen ions and metal ions for sites occurs both on or within biomass (Gadd, 1986); thus cadmium displacement from biomass by hydrogen ions does not necessarily suggest accumulation occurs extracellularly.

3.2.3 Effect of increasing concentration of biomass on cadmium uptake

The binding of cadmium within a 1mg/l solution by different amounts of biomass was investigated for three bacterial species, namely A. viscosus, P. putida and P. cruciviae. Figures 20-21 show the effect of increasing biomass concentration on the percentage of cadmium accumulated. As the amount of biomass present in solution increases, the percentage of cadmium bound within solution increases in a manner suggesting a first order process. As the level of biomass in solution increases the specific binding capacity of the biosorbent decreases (Figs.22-23). When the distribution

FIG.20 EFFECT OF BIOMASS CONCENTRATION ON % UPTAKE
IN 1mg/L CADMIUM BY PS.PUTIDA

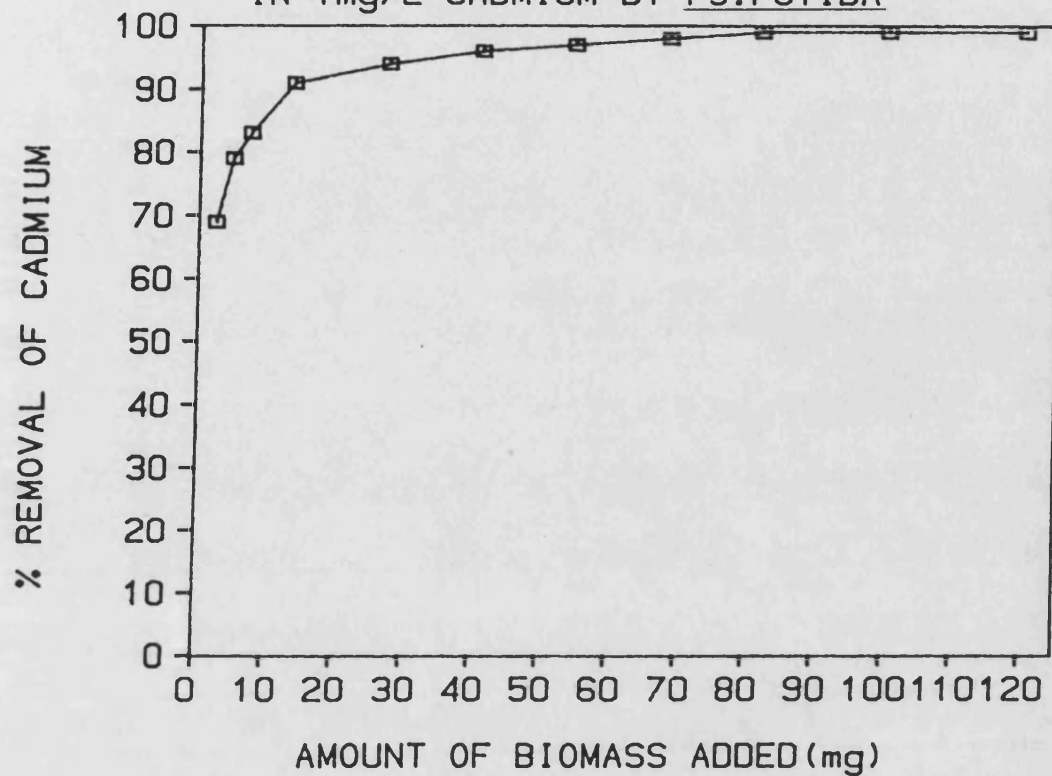


FIG.21 EFFECT OF BIOMASS CONCENTRATION ON % UPTAKE
IN 1mg/L CADMIUM BY PS.CRUCIVIAE

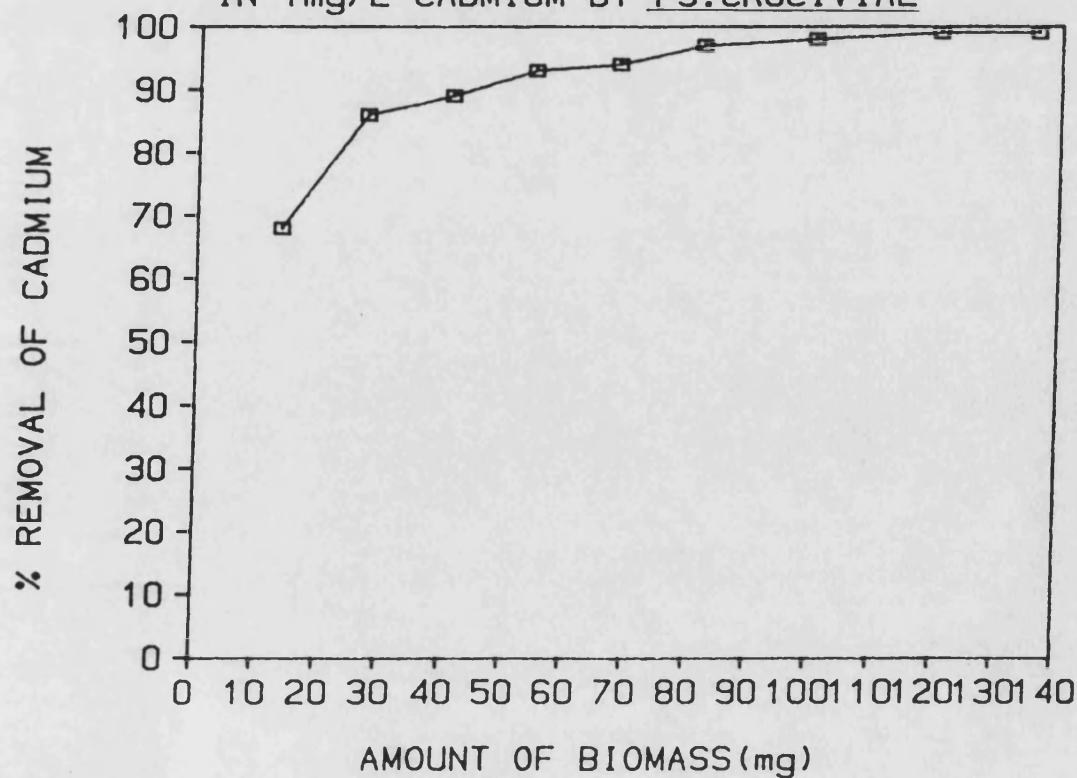


FIG.22 EFFECT OF BIOMASS CONCENTRATION ON BINDING CAPACITY OF A.VISCOSUS AND PS.CRUCIVIAE

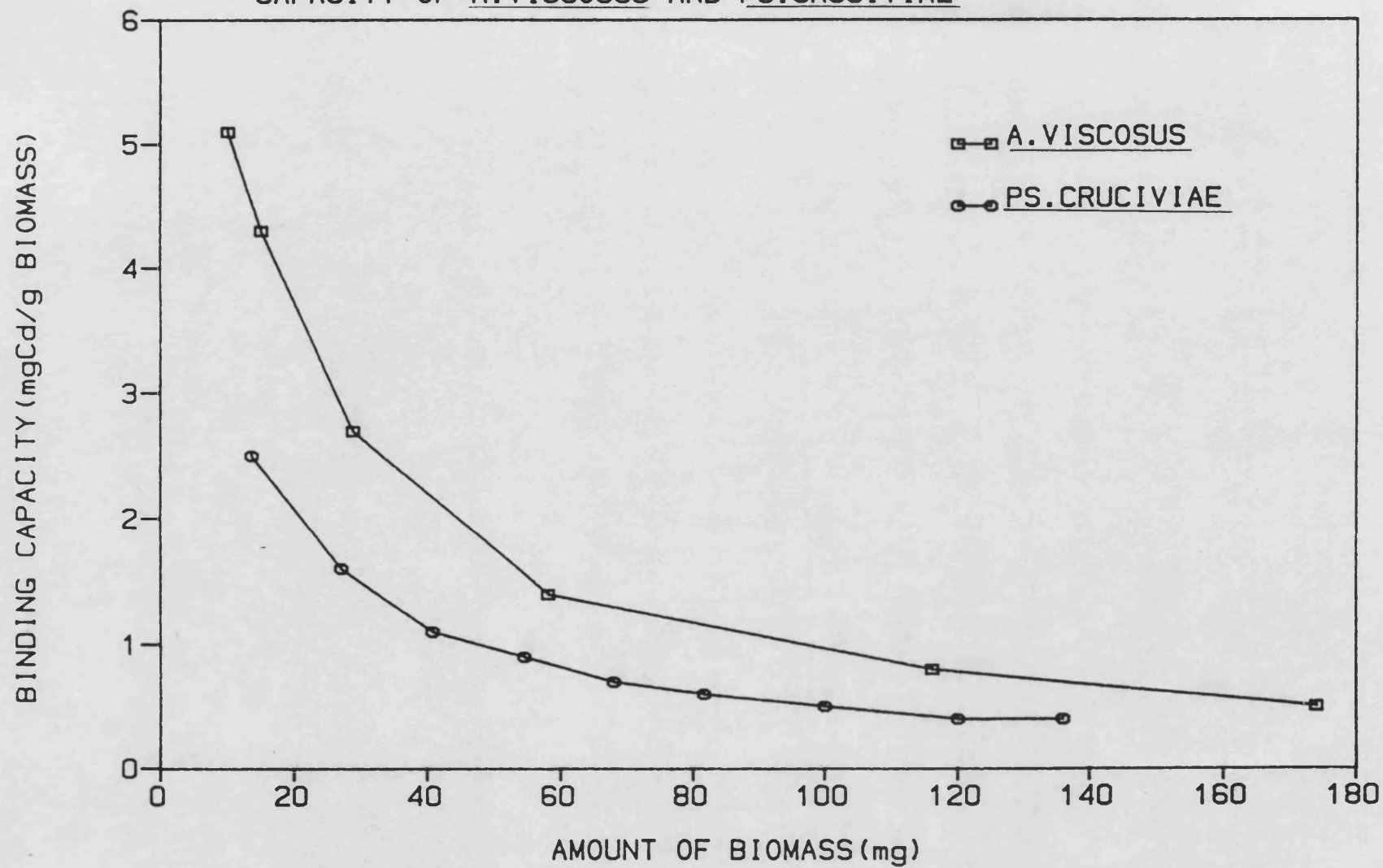


FIG.23 EFFECT OF BIOMASS CONCENTRATION ON BINDING CAPACITY OF PS.PUTIDA IN 1mg/L CADMIUM SOLUTION.

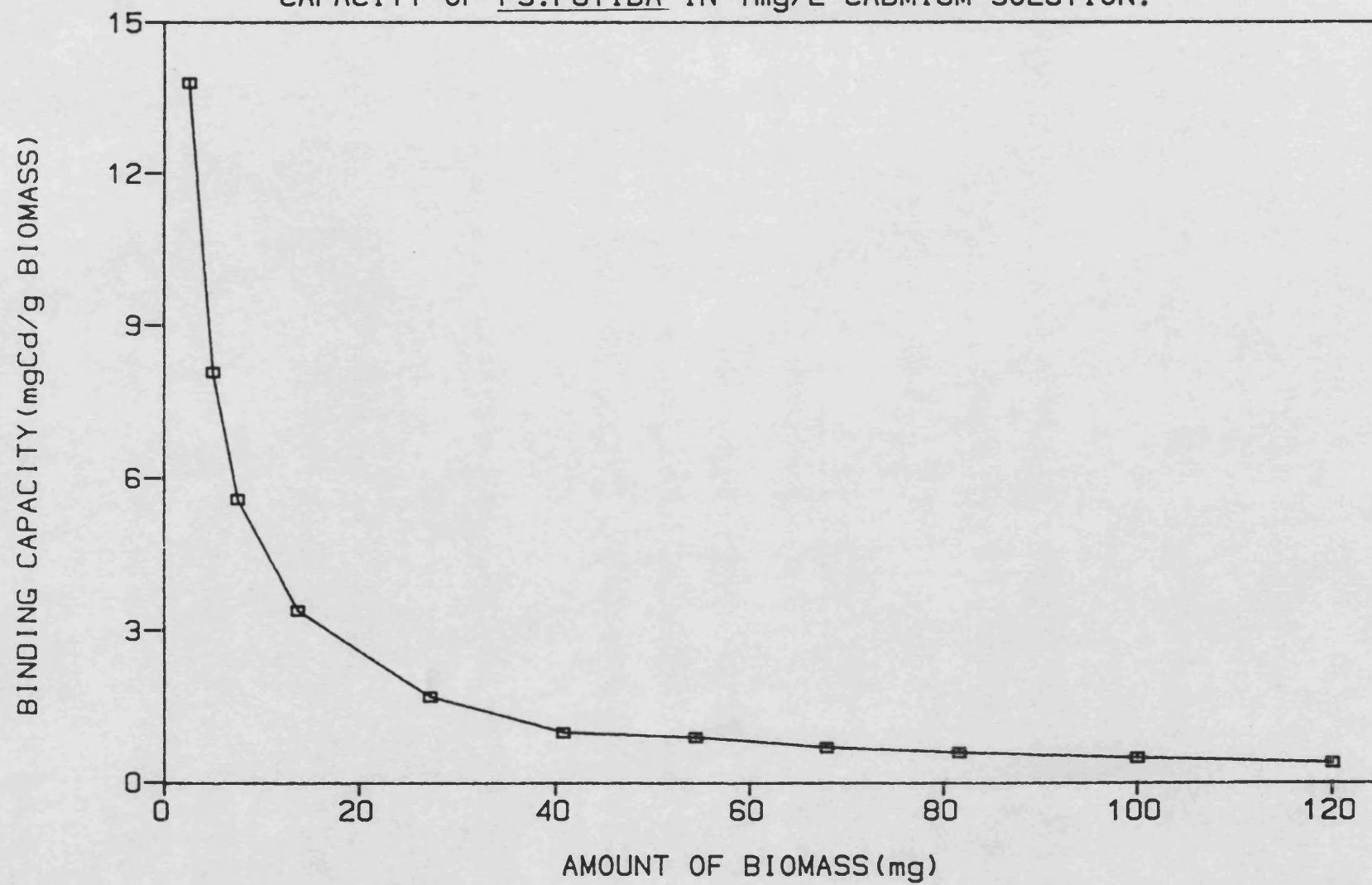
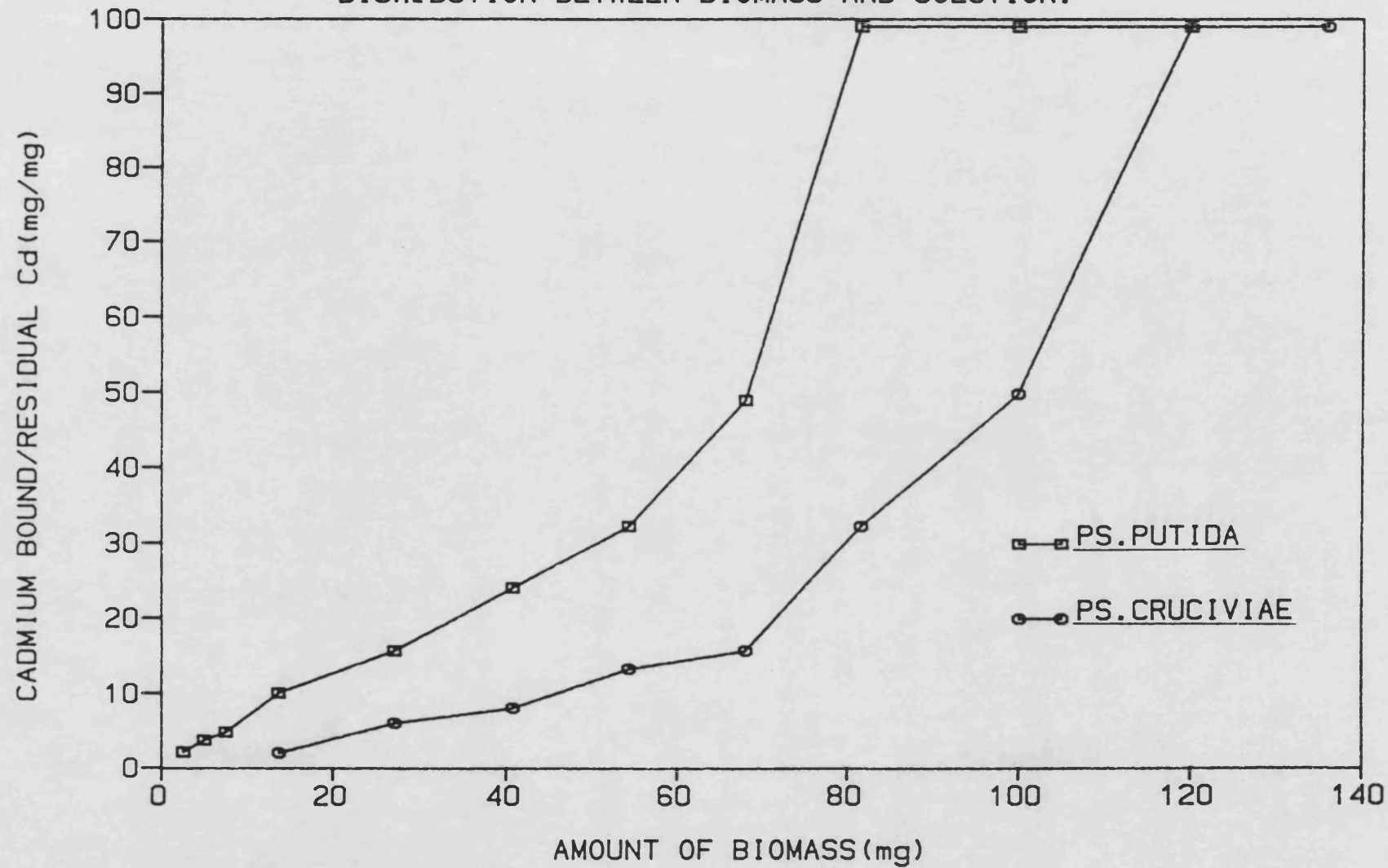


FIG.24 EFFECT OF BIOMASS CONCENTRATION ON CADMIUM DISTRIBUTION BETWEEN BIOMASS AND SOLUTION.



of cadmium between biomass and solution is considered (Figure 24), P.putida is obviously more efficient at binding cadmium than P.cruciviae, which may indicate either some difference in sites, or in mechanisms of cadmium uptake, between the two biosorbents.

Thus the definition of uptake can determine the way in which cadmium uptake appears to be influenced by biomass concentration. If the binding capacity of the biosorbent (mg cadmium per gramme biomass) is used then, as found by Kiff and Little (1986) and de Rome and Gadd (1987), cadmium accumulation is inversely proportional to the biomass concentration (Figures 22,23). However, at any particular cadmium concentration, as the biomass concentration increases the proportion of cadmium bound increases (Figures 20-21).

3.2.4 Effect of state of biomass on biosorption

Throughout the course of this project, live biomass was used for removal of cadmium from solution. To ascertain whether any change in the biosorptive capacity of the biomass occurred when the biomass was non-viable, a sequence of experiments were carried out to compare biosorptive capacity of the same amount of biomass whether viable or non-viable. The species used were rendered non-viable by exposure to 10% formaldehyde or autoclaving at 121°C. In addition, P.cruciviae and

P.putida were also placed in an ultrasonic bath both to kill and disrupt the cells.

Figures 25 to 29 are adsorption isotherms which illustrate the biosorptive capacity of live, autoclaved formaldehyde treated and ultrasonically treated biomass. For A.viscosus (Figure 25) isotherms are very similar for autoclaved and live biomass, although live biomass appeared to be consistently slightly better at cadmium removal. Thus some cadmium biosorption by A.viscosus would appear to be associated with an active process, but only to a very small degree: for example, 16% of binding capacity at 50mg/l cadmium. Formaldehyde treated A.viscosus biomass within the lower initial cadmium concentrations exhibited little difference in binding capacity to that of live biomass, but at higher concentrations the difference was more significant. A similar effect was noted with yeast (Saccharomyces cerevisiae) by Strandberg et al (1981), who proposed that formaldehyde treatment might enhance uranium biosorption by S.cerevisiae by suppressing the amount of positive charge at ionically charged sites on the cell wall, providing more sites for biosorption of metal cations. The same effect is observed for formaldehyde treated A.globiformis biomass (Figure 26), which is more effective at cadmium biosorption than live and autoclaved biomass. For A.globiformis the margin of

FIG.25 UPTAKE OF CADMIUM BY 30mg OF AUTOCLAVED, LIVE AND FORMALDEHYDE TREATED A.VISCOSUS

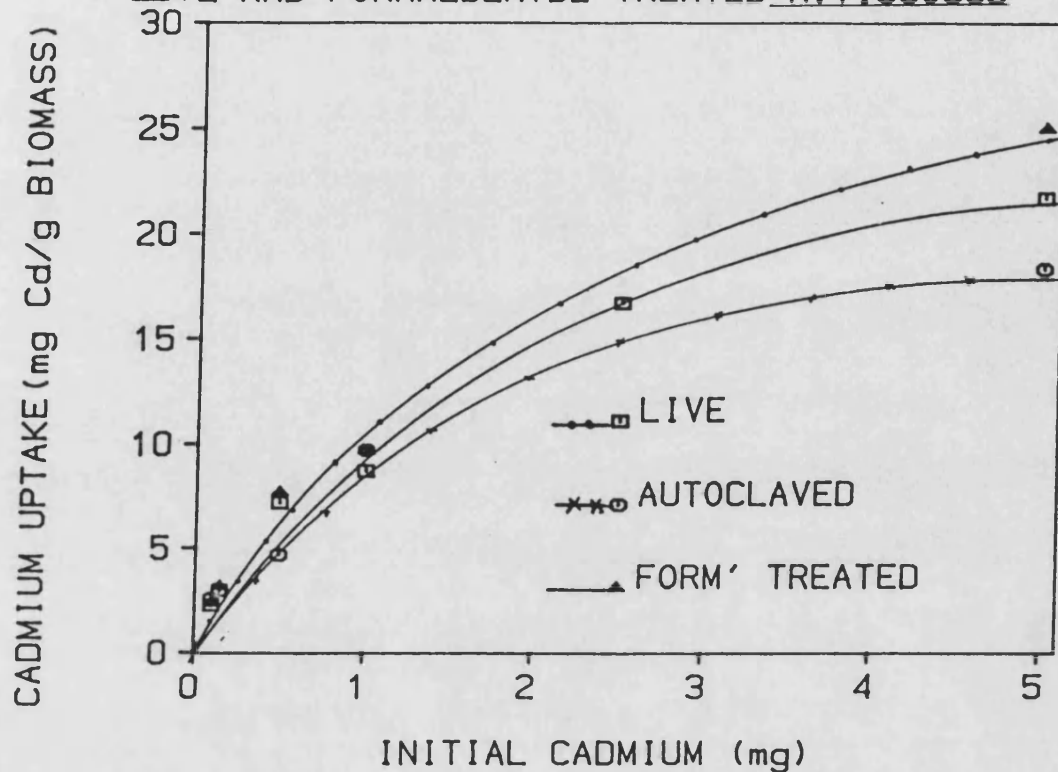
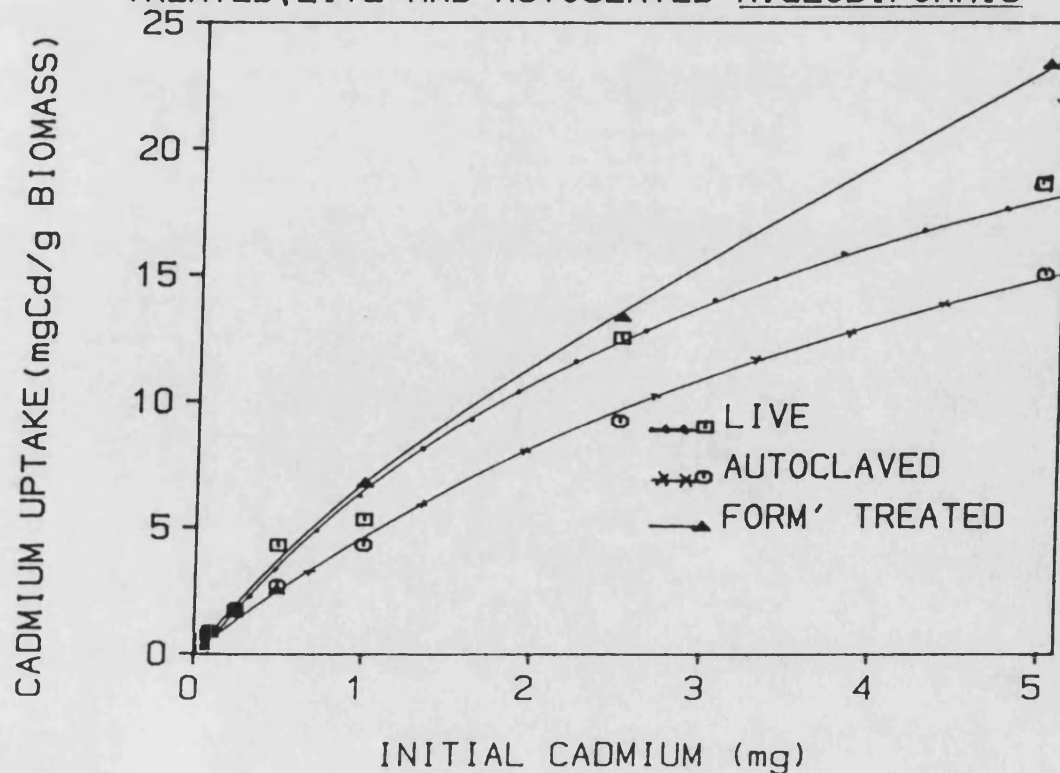


FIG.26 UPTAKE OF CADMIUM BY 30mg OF FORMALDEHYDE-TREATED, LIVE AND AUTOCLAVED A.GLOBIFORMIS



difference between live and autoclaved biomass is quite marked suggesting that part of A.globiformis' binding capacity is dependent on metabolic processes (representing 36% of uptake at 50mg/l initial cadmium). From Figure 27 it may be seen that P.putida live biomass was generally most effective at biosorption closely followed by ultrasonically disrupted cells, then autoclaved cells and finally formaldehyde treated cells. This order of efficiency of cadmium uptake was repeated with the other Pseudomonas sp. investigated, P.cruciviae (Figure 28). Compared to autoclaved biomass, active uptake of cadmium at 50mg/l was 4 and 9% of bound cadmium by P.putida and P.cruciviae respectively.

For K.aerogenes 9128 live and formaldehyde treated biomass gave very similar isotherms with autoclaved cells being less effective than the latter two biomass types. At 50mg/l initial cadmium binding capacity of autoclaved biomass was 8% less than that of live biomass.

In all cases any differences in cadmium binding by the biomass types of the same bacterial species were generally more apparent at higher initial cadmium concentrations, when more of the available binding sites were being filled.

FIG.27 Cd UPTAKE BY 30mg OF ULTRASONICALLY TREATED, FORMALDEHYDE TREATED, AUTOCLAVED AND LIVE PS.PUTIDA

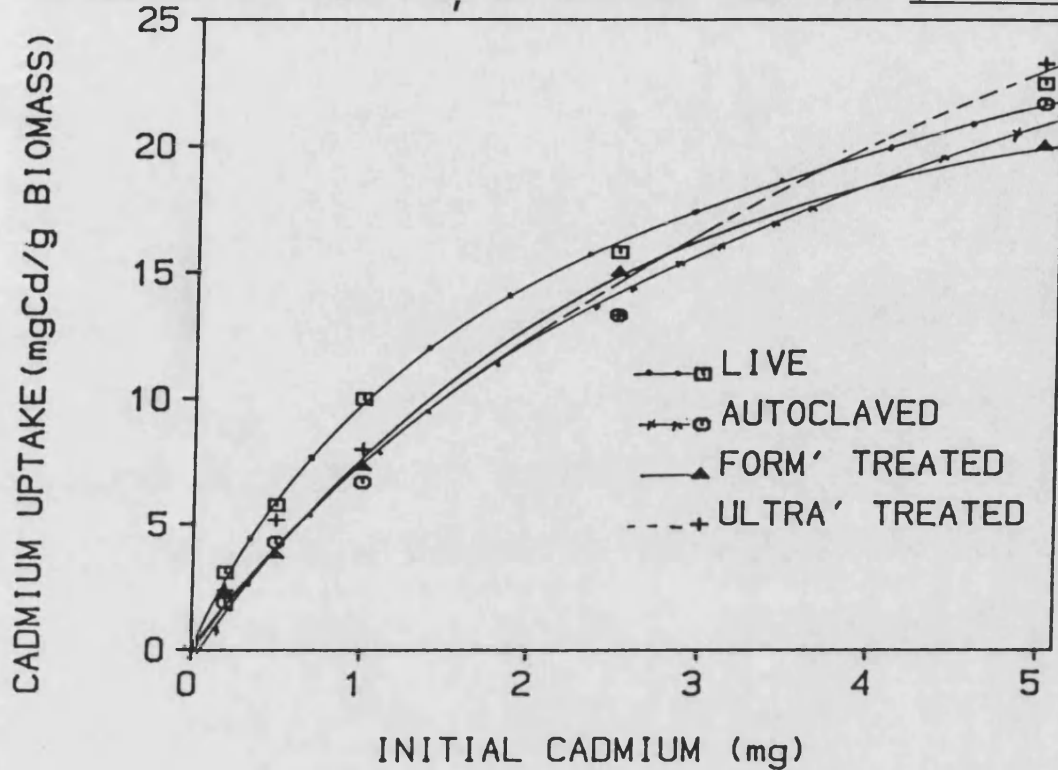
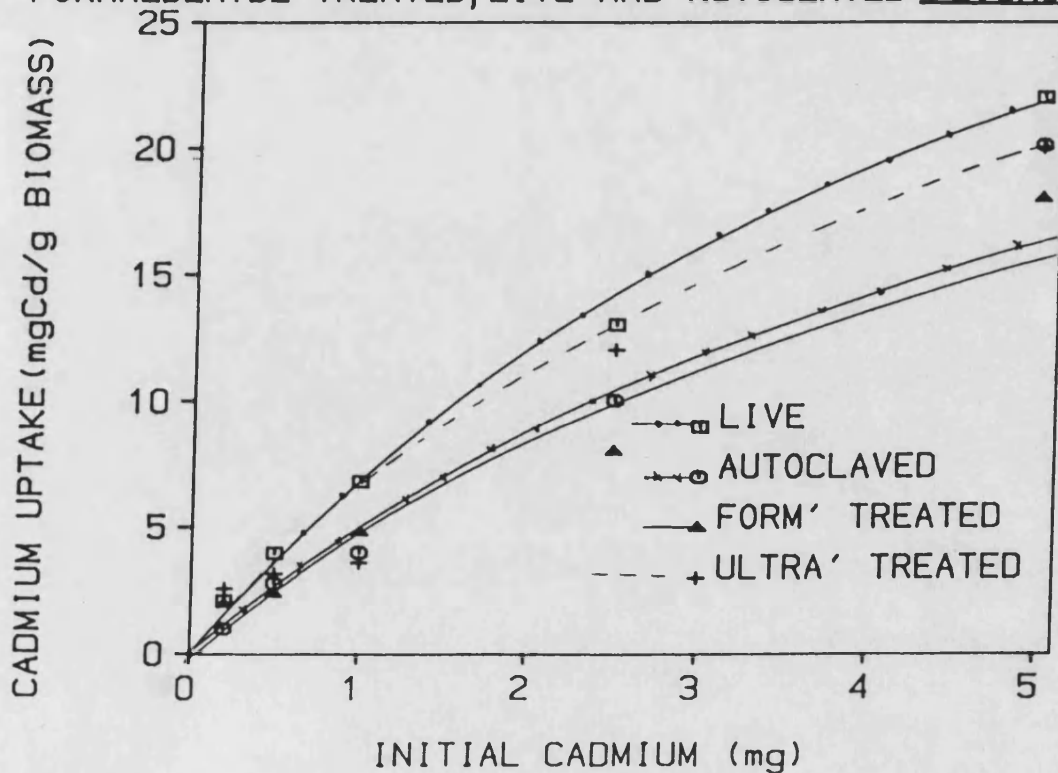


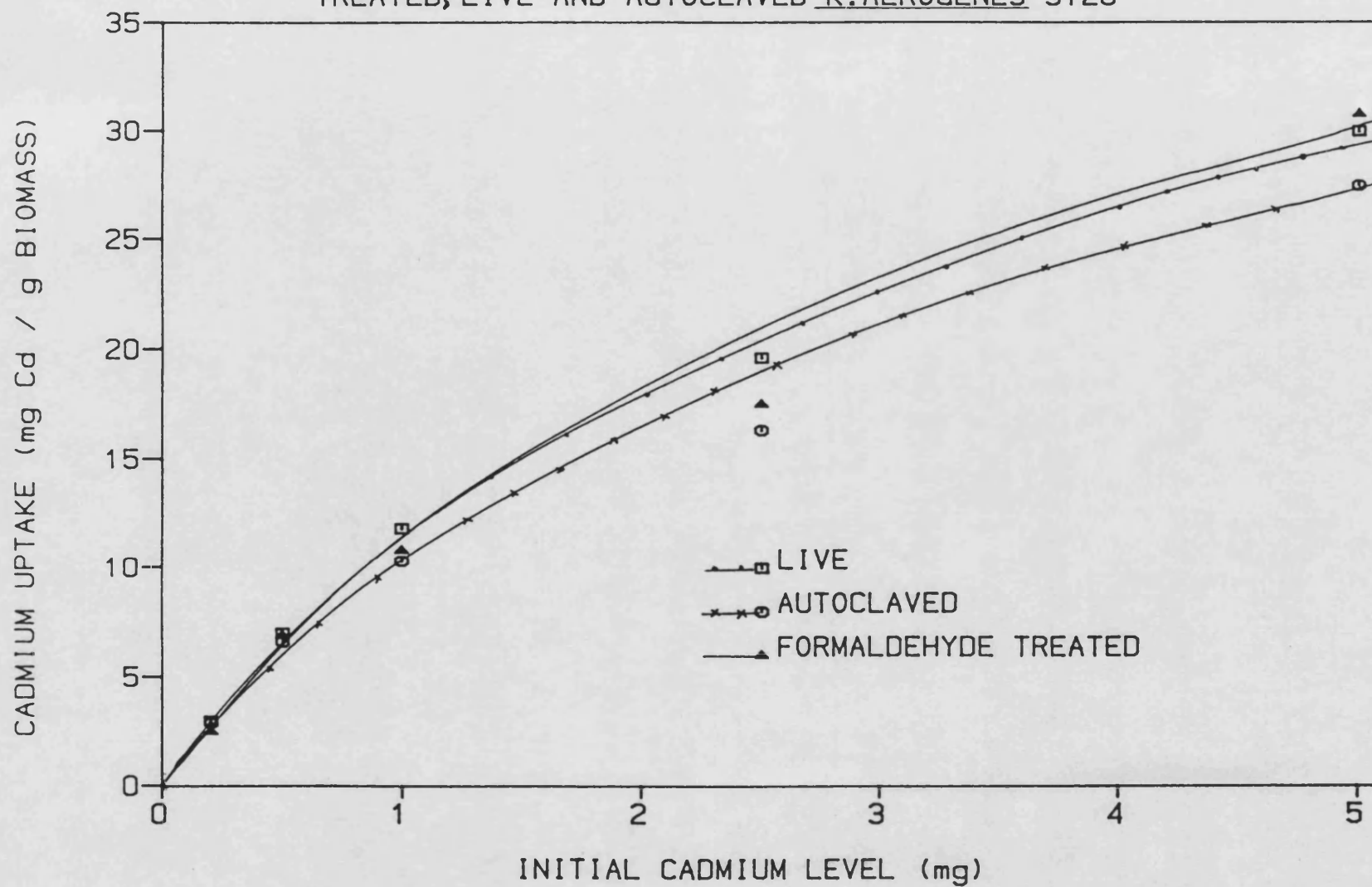
FIG.28 Cd UPTAKE BY 30mg OF ULTRASONICALLY TREATED, FORMALDEHYDE TREATED, LIVE AND AUTOCLAVED PS.CRUCIVIAE



Formaldehyde treatment appeared to either enhance cadmium accumulation or have little effect. In the case of P.putida and P.cruciviae formaldehyde treatment gave no better uptake of cadmium than autoclaved biomass, yet ultrasonically disrupted cells bound more cadmium than the latter two types at higher initial cadmium levels. The work of Horitsu (1978) and Strandberg et al (1981) indicates that metal binding by Pseudomonas sp. is predominantly intracellular. This concurs with the evident uptake of cadmium exhibited by P.cruciviae and P.putida by ultrasonically disrupted cells, as at lower cadmium concentrations most available cadmium could be bound to sites on the cell exterior; however, at higher levels of cadmium such sites would become saturated and as the bulk of binding sites for metal by pseudomonads are within the cell, disrupted cells would bind more cadmium as their interiors lie exposed. Horitsu (1978) determined that 80% of metal bound by P.ambigua was bound within the cell which may explain why formaldehyde treatment did little to enhance cadmium uptake by pseudomonads in this investigation. They would appear to have few binding sites on the cell surface that might be affected by formaldehyde in the way that surface binding of uranium to Saccharomyces cerevisiae was enhanced according to Strandberg et al (1981).

The binding of cadmium to K.aerogenes 9128 would appear

FIG.29 CADMIUM UPTAKE BY 30mg OF FORMALDEHYDE-TREATED, LIVE AND AUTOCLAVED K.AEROGENES 9128



to occur on the cell exterior to some degree as formaldehyde treatment did in fact give biomass enhanced uptake over that exhibited by autoclaved biomass, but not greater than that of live biomass. Formaldehyde treatment of A.viscosus and A.globiformis improved cadmium binding over that of live biomass beyond 5mg/l initial cadmium. This improvement in cadmium uptake by both formaldehyde treated Arthrobacter sp. over that of autoclaved biomass suggests that some cell surface binding of cadmium occurs, assuming again that this effect predominates at the cell surface rather than within the cell.

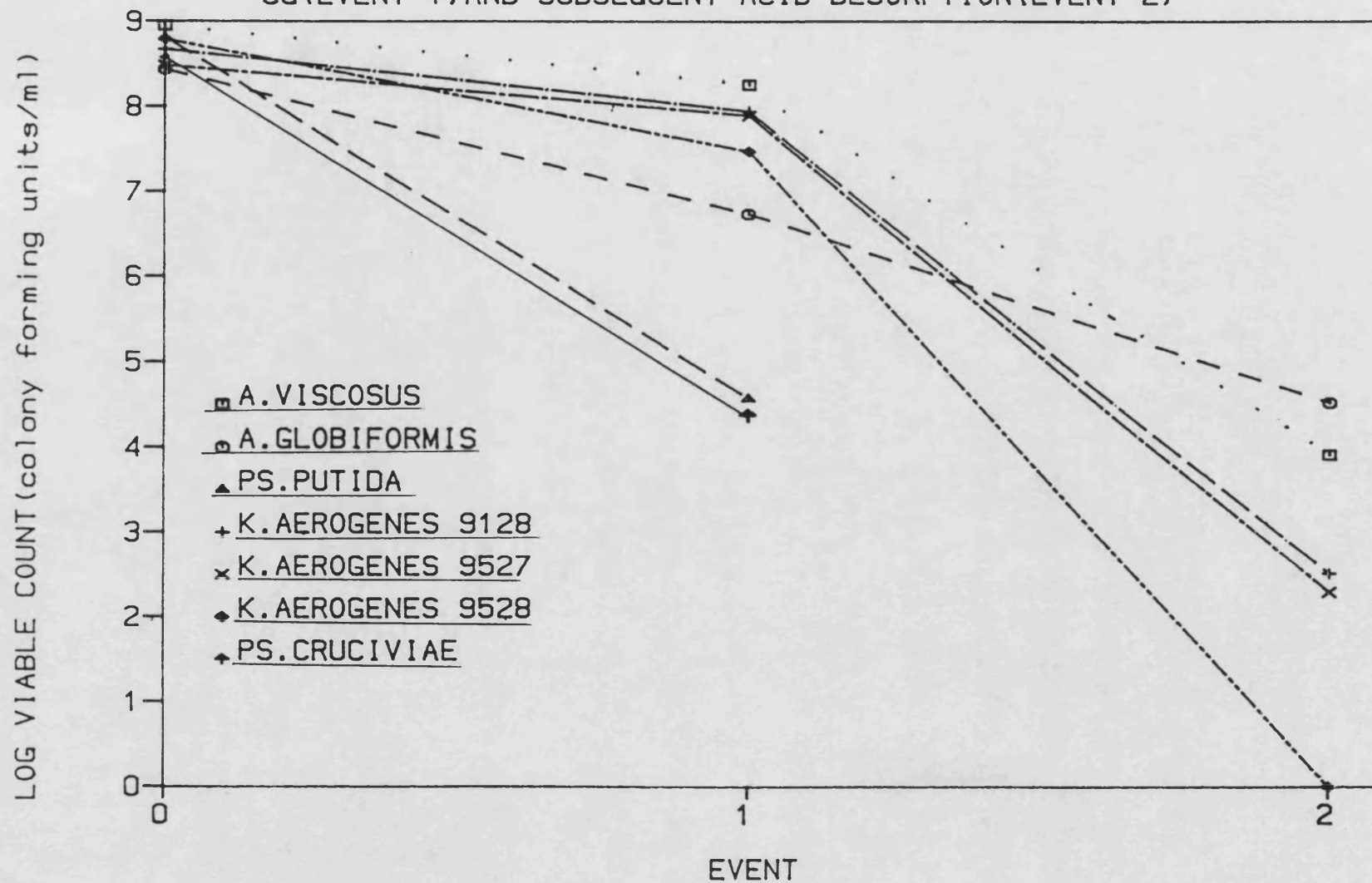
Overall it is apparent that in all cases most of the cadmium bound by the biosorbents used is accumulated whether the cells are viable or non-viable. A.globiformis exhibits the greatest degree of active cadmium accumulation at approximately one third of its capacity at 50mg/l; for the other bacterial species the amount of active cadmium accumulation was almost negligible in comparison.

3.2.5 Effect of cadmium biosorption on cell viability

Live cells were used in most biosorption trials carried out during this course of study. With regard to the application of a live biomass to a continuous treatment process, the effect of cadmium biosorption on cell survival and, in addition, the effect of subsequent acid desorption of cadmium on cell survival needed to be assessed.

Figure 30 illustrates the decline in the number of colony forming units (c.f.u.) following ten minutes exposure of cells to cadmium solution and then subsequent cadmium desorption by 0.1M hydrochloric acid over 15 minutes. The viability of P.cruciviae and P.putida biomass was most affected by exposure to cadmium. Table 6 presents the reduction in viable count following acid desorption expressed in terms of logarithmic units. The count was presented in this manner to clarify the trends illustrated in Figure 30. The two pseudomonads had their viable counts both reduced by over 4 \log_{10} units by exposure to 100mg/l cadmium, making them far more susceptible to cadmium toxicity than any of the other bacterial strains. K.aerogenes strains 9128 and 9527 both had similar \log_{10} reductions in their viable count whereas K.aerogenes 9528 had a \log_{10} reduction almost twice that of the other two Klebsiella strains. A.viscosus was similarly

FIG.30 VIABLE COUNT FOLLOWING EXPOSURE TO 100mg/L Cd (EVENT 1) AND SUBSEQUENT ACID DESORPTION (EVENT 2)



resistant to cadmium toxicity as K.aerogenes 9128 and 9527. A.globiformis was over twice as susceptible to cadmium as A.viscosus, but not affected as much as the two Pseudomonas strains. Exposure to 0.1M hydrochloric acid heavily reduced the viable cell populations of all the bacterial strains. Most acid resistant was A.globiformis.

Acid desorption was not carried out on either Pseudomonas strain. All other bacterial strains washed with acid were affected far more than A.globiformis; in terms of \log_{10} units, A.viscosus and K.aerogenes 9128 and 9527 were over twice as badly affected by acid. K.aerogenes 9528 again proved to be less robust than the other two Klebsiella strains, its viable count being reduced by acid treatment three times more than that of A.globiformis when expressed as \log_{10} units. In all cases acid desorption resulted in a greater reduction in the viable cell population than cadmium toxicity did at 100mg/l cadmium.

If the bulk of metal bound by P.cruciviae and P.putida may be presumed to be bound intracellularly, as is the case with P.aeruginosa (Strandberg et al, 1981), then this would explain the greater toxicity of cadmium for the two pseudomonads. Intracellularly bound cadmium would be much more likely to interfere with cell

TABLE 6: Reduction of Viable Count (C.F.U./ml) by Cadmium Exposed [1] then Acid-Washed [2] Biomass

ORGANISM	\log_{10} (initial count/ count after Cd exposure)	\log_{10} $\left(\frac{\text{count after Cd exposure}}{\text{count after acid wash}} \right)$
<u>A.viscosus</u>	0.70	4.32
<u>A.globiformis</u>	1.69	2.20
<u>P.putida</u>	4.23	-
<u>P.cruciviae</u>	4.63	-
<u>K.aerogenes</u> 9128	0.71	5.46
<u>K.aerogenes</u> 9527	0.60	5.60
<u>K.aerogenes</u> 9528	1.30	7.48

[1] 50ml 100mg/l cadmium 20°C; ph 7 ± 0.5 . Exposed to solution for 10 minutes

[2] 0.1M HNO₃(99) 20°C. Exposure time 15 minutes.

metabolism. Strandberg et al (1981) also found that only 44% of the P.aeruginosa population bound cadmium to the degree that it was visible by E-M, thus surviving cells in the pseudomonad population may be those that did not bind cadmium intracellularly. Of the two Arthrobacter species, A.viscosus was more cadmium tolerant. This may in part be due to the production of extracellular polysaccharide which can chelate cadmium; however experimentation has shown that the amount of polysaccharide excreted from the biomass over the period monitored was not responsible for the total difference in metal uptake between the two Arthrobacters at low cadmium concentrations (see section 3.2.6). Both Arthrobacter strains were far less susceptible to cadmium than the pseudomonads. This might be because the Arthrobacter strains do not bind their cadmium intracellularly, or have some mechanism for preventing cadmium ions from exerting their full toxicity. In the latter respect it is worth noting that Arthrobacter species have been found in uranium ores (Updegraff et al, 1971) and uranium mill tailings (Miller et al, 1987) thus appearing quite resistant to heavy metal toxicity. Arthrobacter sp. are known to be quite robust and inhabit a variety of environments over quite a wide range of pH (Keddie and Jones, 1983), hence it might be expected that the effect of acid desorption would be least on A.globiformis and A.viscosus.

Of the three K.aerogenes strains, strain 9528 fared worst with regard to drop in viable cell population following both exposure to cadmium and acid desorption. This is likely to be the result of the lack of an extracellular polysaccharide capsule surrounding strain 9528, which both strains 9128 and 9527 (strain 9527 selected as it is the same serotype as 9528) possessed. Thus it would appear that presence of a polysaccharide capsule enhances the survival of K.aerogenes with regard to metal toxicity and acid desorption. This is confirmed with regard to cadmium toxicity by Pickett and Dean (1976) who found that K.aerogenes was more sensitive to cadmium under conditions of glucose limitation, when no such extracellular capsule is formed. The K.aerogenes strains were markedly less susceptible to cadmium toxicity than the Pseudomonas strains. This is possibly due to the physiological adaptation that K.aerogenes exhibits to cadmium as elucidated by Aiken et al (1982) who found that cadmium exposed continuous culture soon became quite insensitive to cadmium through precipitating the cadmium as cadmium sulphide at the cell surface. However, despite this mechanism survival was significantly enhanced by possession of an extracellular polysaccharide capsule. In addition Aiken et al (1984) found a K.aerogenes strain capable of detoxifying cadmium in continuous culture by precipitating it with

inorganic phosphate accumulated within the cell. Thus at least three mechanisms are known by which K.aerogenes mediates cadmium toxicity. This is significant as a combination or one of these might be responsible for cadmium uptake of a strain used for metal recovery, and the mechanism of accumulation is a factor determining the suitability of the bacteria for use.

3.2.6. Effect of cell produced polysaccharide on cadmium biosorption

Of the bacteria investigated, several were selected because they were capable of producing extracellular polysaccharide.

Of A.viscosus and A.globiformis, the former is capable of excreting quite high levels of polysaccharide and the latter was studied as a comparison organism not capable of production of copious quantities of polysaccharide in the nutrient medium employed. Of the K.aerogenes strains employed, two produced an extracellular polysaccharide capsule attached to the cell (strains 9128 and 9527) whereas strain 9528, selected for comparison, did not. Neither of the two Pseudomonas strains employed were capable of production of capsular or extracellular polysaccharide under the experimental conditions maintained. The isotherms of Figure 8 show that polysaccharide forming K.aerogenes strains 9128 and

9527 are more effective at binding cadmium than non-polysaccharide capsule producing strain K.aerogenes 9528. Similarly, A.viscosus, an extracellular polysaccharide excreter, is generally more effective than A.globiformis. However, in both these cases, the difference between the proportion of cadmium removed by polysaccharide forming organisms such as A.viscosus and K.aerogenes strains 9128 and 9527 and the comparison microorganisms, A.globiformis and K.aerogenes 9528 respectively, decreases as the initial cadmium concentration increases (Figures 6-8, Table 1.1). Despite this, for the microorganisms compared in this project, polysaccharide production appeared to enhance cell survival and cadmium binding over a wide concentration range.

Using A.viscosus, an attempt was made to determine what proportion of cadmium immobilised by the biomass was bound to the excreted polysaccharide. Under standard experimental conditions (see Materials and Methods) the polysaccharide excreted from 30mg of A.viscosus was extracted and its binding capacity evaluated and compared to that of the stripped biomass with 50ml of 2mg/l cadmium solution. The recovered polysaccharide was found to bind 5% of the cadmium present, whereas A.viscosus cells bound 80% of the available cadmium. In comparison 30mg of A.globiformis cells bound only 34% of

available cadmium. These figures represent an average from duplicate experiments. Although the polysaccharide recovered from A.viscosus biomass under these conditions bound only a fraction of the cadmium available, in terms of specific binding capacity it was a more efficient biosorbent than the cells themselves, as only a small amount (i.e. 1.2mg) dry mass on average over the two experiments) was recovered. Thus the binding capacity of the recovered A.viscosus polysaccharide under these conditions was 0.66, that of the cells 0.54, and that of A.globiformis cells, 0.23 (mg cadmium per gramme biomass). It would appear from this data that polysaccharide excreted by A.viscosus does not alone account for the difference in biosorptive capacity between A.viscosus and A.globiformis. However, this may not provide a true picture of the distribution of cadmium between A.viscosus and its extracellular polysaccharide. Extraction of extracellular polymer from solution might not remove all of the polysaccharide present as some might still remain attached to the cell (Rudd et al, 1982). Alternatively, removing polysaccharide might expose previously unexposed sites on the cell (Sterritt and Lester, 1986).

The structures of the extracellular polysaccharides of A.viscosus and K.aerogenes strain 9527 are known. The basic constituents of A.viscosus NCIMB 9728 (NRRC B-

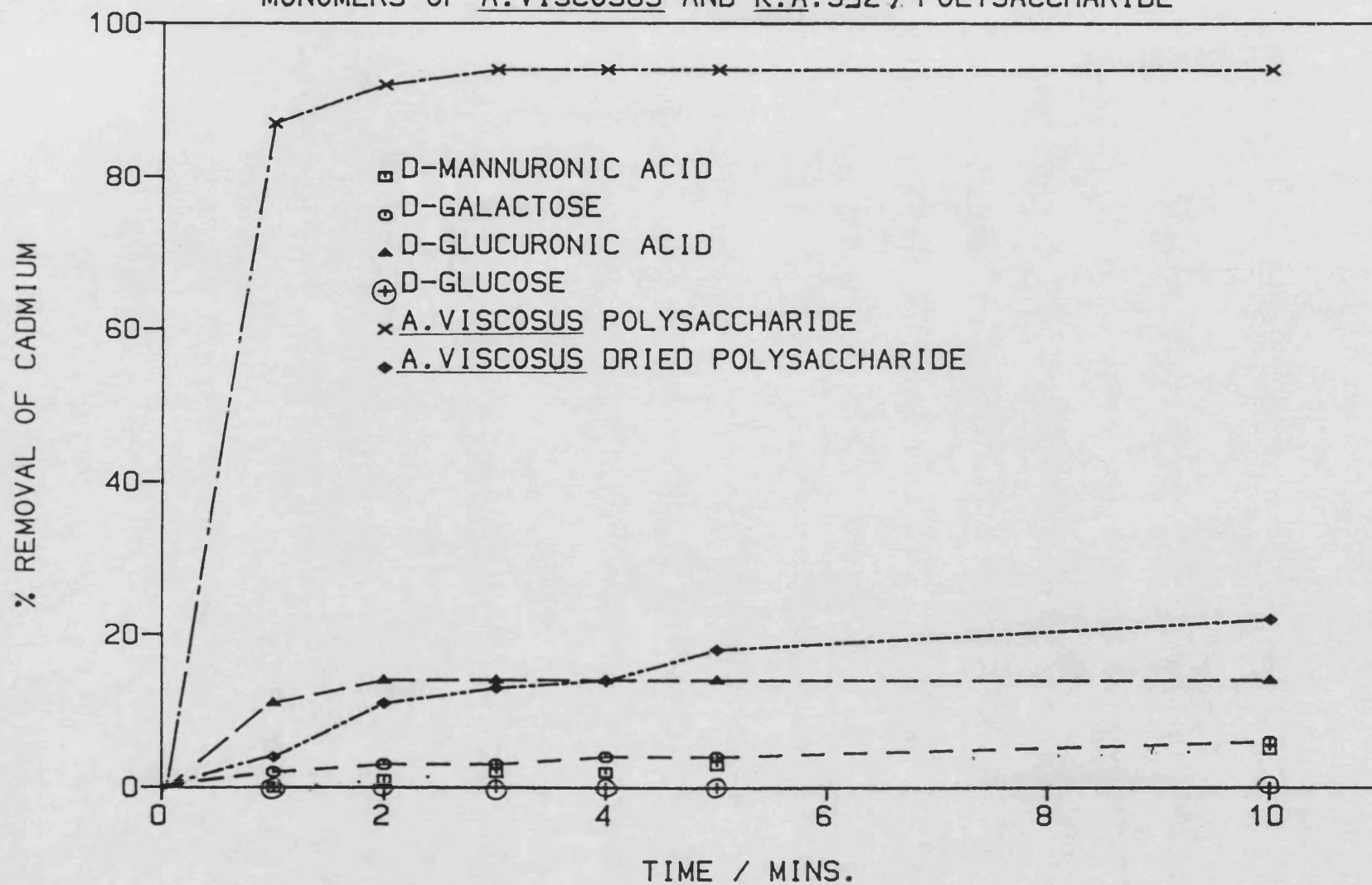
1973) exopolysaccharide are D-glucose, D-galactose and D-mannuronic acid (Sloneker et al, 1968), present in equimolar proportions and joined linearly as repeated trisaccharide units. K.aerogenes 9527 and 9128 are serotype 8; the structure of the exopolysaccharide corresponding to this serotype has been determined and its constituent monomers identified as D-galactose, D-glucose and D-glucuronic acid, present in the ratio 1:2:1 respectively (Dudman and Wilkinson, 1956). A series of trials was accomplished in which the binding capacity of the constituent monomers of A.viscosus NCIMB 9728 exopolysaccharide was determined and compared to that of purified received polysaccharide. The kinetics of biosorption for the polysaccharide constituents and air dried and non air dried A.viscosus 9728 polysaccharide are illustrated in Figure 31. The specific binding capacity of each constituent monomer of A.viscosus 9728 and K.aerogenes 9527 exopolysaccharide are presented in Table 7. Of the single components of A.viscosus 9728 polysaccharide, the most effective at complexing with cadmium was D-galactose, which was marginally better than D-mannuronic acid. D-glucose was not found to be capable of removing cadmium from solution under these conditions. A mixture of these component monomers was no more efficient at complexing cadmium than D-mannuronic acid alone. However, two samples of purified polysaccharide of A.viscosus 9728

TABLE 7: Specific binding capacity at equilibrium of constituent monomers of *K.aerogenes* 9527 and *A.viscosus* 9728 exopolysaccharide and binding by purified and recovered *A.viscosus* polysaccharide

BIOCHEMICAL	Amount Present (mg)	Specific binding capacity at equilibrium (mgCd/g biosorbent)	% Cadmium removed at equilibrium
D-Glucose	30	0.00	0
D-Galactose	30	0.10	6
D-mannuronic acid	30	0.09	5
D-gluc uronic acid	30	0.23	14
D-Glucose + D-Galactose + D-mannuronic acid	10 mg of each (30 total)	0.09	5
D-Glucose + D-Galactose + D-gluc uronic acid	10 mg of each (30 total)	0.33	20
<i>A.viscosus</i> dried polysaccharide	30	0.37	22
<i>A.viscosus</i> undried polysaccharide	30	3.28	94

NB: 50ml 1mg/l cadmium; pH 7 \pm 0.5; 20°C.

FIG.31 KINETICS OF CADMIUM UPTAKE BY CONSTITUENT MONOMERS OF A.VISCOSUS AND K.A.9527 POLYSACCHARIDE



were both more effective biosorbents than equivalent masses of their constituent monomers or a mixture of said monomers. Of the two polysaccharide samples, one was an air dried sample, which was added directly to 50ml of cadmium solution. The dried polysaccharide was far less efficient at cadmium binding than the non-dried material and in fact the dried material continued to remove cadmium from solution for at least two hours. The actual figure quoted in Table 7 for cadmium biosorption by dried A.viscosus polysaccharide is thus not its biosorptive capacity at equilibrium, but the point in time (10 mins) where cadmium uptake by all the other biochemicals used had reached equilibrium.

A sample of purified polysaccharide was redissolved in a known volume of water, and its dry weight per unit volume determined so that an equivalent weight of undried polysaccharide could be used in a metal biosorption trial without air drying the polysaccharide. The undried polysaccharide was exposed to a larger volume (100 mls) of 1mg/l cadmium than the dried polymer , but still removed over four times as much cadmium from the solution. Drying A.viscosus polysaccharide would appear to reduce its capacity for binding cadmium, perhaps due to some alteration of the structure of polysaccharide. Once re-introduced into aqueous solution, dried polysaccharide was observed visually to swell gradually in volume, and as previously stated, gradually bound

more cadmium over an extended period of time (Scott and Palmer, 1988) . The polysaccharide constituent monomers alone, and in combination as a mixture in solution, were not as effective at cadmium binding as the A.viscosus polysaccharide itself. The overall structure of the polysaccharide itself provides more sites for cadmium complexation than could be accounted for simply by the binding capacity of constituent monomers alone.

Of the constituent monomers of K.aerogenes serotype 8 capsular polysaccharide glucuronic acid was the most effective at cadmium complexation. However, a mixture of the three constituent monomers was more efficient at cadmium binding than any single monomer alone (Table 7). The biosorptive capacity of the exopolysaccharide of the exopolysaccharide of A.viscosus 9728 was then compared with that of A.viscosus cells in terms of equivalent masses of each. The biosorptive capacity of cells and polysaccharide was also determined following one loading sequence, i.e. cells and polysaccharide had their biosorptive capacity evaluated after already being exposed to cadmium solution - but not subsequently desorbed of cadmium. These results are presented in Table 8.

TABLE 8: Biosorptive Capacity of Cells and Polysaccharide of *A.viscosus* 9728 on Exposure to 100ml 1mg/l Cadmium

BIOSORBENT	Amount Present (mg dry weight)	% Cadmium removed	Biosorptive Capacity (mgCd/g biosorbent)
<u><i>A.viscosus</i></u> cells	29	77	2.66
Polysaccharide	29	94	3.28
* Pre-exposed cells	29	69	2.40
* Pre-exposed polysaccharide	19	72	3.79

NB: Cadmium solution pH 7 ± 0.5 ; 20 C.

*Pre-exposed to 100ml 1mg/l cadmium, then recovered by centrifugation prior to next exposure to cadmium solution.

Comparison of equivalent masses of polysaccharide and A.viscosus cells (Table 8) shows that the polysaccharide was a more effective biosorbent than the cells (it should be noted that in these trials polysaccharide was not recovered from the cells used). Despite the fact that less polysaccharide was used for cadmium uptake evaluation following previous exposure to cadmium than A.viscosus cells, the polysaccharide again removed a greater proportion of the cadmium present.

Thus, the biosorptive capacity of the polysaccharide produced by A.viscosus is greater than that of the A.viscosus cells themselves, but would not appear to contribute greatly to the observed cadmium uptake of this biomass under the conditions employed.

3.2.7 Effect of other metal ions on cadmium biosorption

The effect of the presence of other metal ions on the binding of cadmium by exopolysaccharide producing strains A.viscosus and K.aerogenes 9128 was investigated. The uptake of zinc, nickel, lead, copper and cadmium was determined individually for both bacterial strains at 1 and 10mg/l of metal ions and compared to biosorption levels achieved with mixtures of these metal ions.

For comparison biosorption of these metals by

A.globiformis under the conditions described was also determined. Additionally, the biosorption of lead by both these Arthrobacter strains over a range of lead concentrations was determined (Scott, Palmer and Ingham, 1986). Order of decreasing preference (Tables 10 and 11) for accumulation of individual metals at 1 and 10mg/l concentrations was $Pb^{2+} > Cu^{2+} > Cd^{2+} > Ni^{2+} > Zn^{2+}$ for both A.viscosus and K.aerogenes 9128. At 10mg/l, uptake by A.globiformis was similar with the exception that Zn^{2+} was bound more readily than Ni^{2+} . The effect of several mixtures of metal ions on cadmium biosorption was assessed at three levels. The percentage change in cadmium bound was determined as:

- (i) the level of cadmium bound in mixed solution compared to that for the same level of cadmium bound in cadmium solution alone;
- (ii) the level of cadmium bound in mixed solution compared to that level of cadmium alone equivalent to the overall metal concentration
- (iii) the amount of metal bound overall in mixed solution compared to the amount of cadmium bound in a solution of cadmium of the same strength.

Tables 12-14 show these values for equimolar solutions of copper, cadmium and zinc at three different concentrations using A.viscosus, and two concentrations for A.globiformis and K.aerogenes 9128. Table 15

TABLE 10: Biosorption of Metal Ions from 1mg/l solutions at pH 7 ± 0.5 and 20 °C by *A.viscosus* and *K.aerogenes*

ORGANISM	METAL ION				
	Cd ²⁺	Pb ²⁺	Cu ²⁺	Zn ²⁺	Ni ²⁺
<u><i>A.viscosus</i></u> cells	0.072	0.090	0.082	0.070	0.070
<u><i>K.aerogenes</i></u> 9128	0.065	0.088	0.078	0.054	0.064

Results are average of duplicate trials. pH = 7 ± 0.5; 20°C.

* Expressed as mg of metal removed by 30mg of biomass;
100ml of metal solution present (i.e. initially 0.1mg metal present).

TABLE 11: *Biosorption of Metal Ions from 10mg/l solutions at pH 7 + 0.5 and 20°C by
A.viscosus; A.globiformis and K.aerogenes 9128

ORGANISM	METAL ION				
	Cd ²⁺	Pb ²⁺	Cu ²⁺	Zn ²⁺	Ni ²⁺
<u>A.viscosus</u>	0.31	0.90	0.71	0.11	0.12
<u>A.globiformis</u>	0.24	0.87	0.32	0.09	0.04
<u>K.aerogenes 9128</u>	0.36	0.91	0.62	0.17	0.24

Results are the average of duplicate trials.

* Expressed as mg of metal removed by 30mg of biomass;
100ml of metal solution present (i.e. initially 1.0mg metal present).

TABLE 12: *Biosorption of 0.33mg/l each of Cd^{2+} , Cu^{2+} and Zn^{2+} by 30mg *A.viscosus*

METAL IONS			Initial Total metal (mg)	Overall Total Metal bound (mg)	% Reduction [⊕] Cadmium bound	‡% Change in overall amount of metal bound
Cu^{2+}	Cd^{2+}	Zn^{2+}				
0.033	0.0267	0.0211	0.99	0.0808	18	+92

* Expressed as mg of metal bound: pH = 7 ± 0.5 ; 20°C

⊕ Versus 0.3mg/l cadmium solution

‡
 + = Increase in overall metal uptake versus 1mg/l cadmium
 - = Decrease in overall metal uptake versus 1mg/l cadmium

TABLE 13: *Biosorption of Cu²⁺ Cd²⁺ and Zn²⁺ from a solution containing 1mg/l each by 30mg *A.viscosus*; *A.globiformis* and *K.aerogenes* 9128

ORGANISM	METAL ION			Initial Total metal (mg)	Overall Total Metal bound (mg)	†% reduction cadmium bound		‡% change in overall amount of metal bound
	Cd ²⁺	Cu ²⁺	Zn ²⁺			A	B	
<u><i>A.viscosus</i></u>	0.068	0.063	0.078	0.3	0.209	6	59	+27
<u><i>A.globiformis</i></u>	0.0243	0.080	0.037	0.3	0.140	17	84	0
<u><i>K.aerogenes</i> 9128</u>	0.28	0.066	0.007	0.3	0.101	57	82	-35

* Expresses as mg of metal bound. pH 7 ± 0.5; 20°C

† versus, column (A) 1mg/l cadmium, and column (B), 3mg/l cadmium solution

‡ + = % increase in amount of metal bound vs 3mg/l cadmium
 - = % decrease in amount of metal bound vs 3mg/l cadmium

TABLE 14: *Biosorption of Cu^{2+} , Cd^{2+} and Zn^{2+} from a solution containing 10mg/l of each by 30mg *A.viscosus*; *A.globiformis* and *K.aerogenes* 9128

ORGANISM	METAL ION			Initial Total metal (mg)	Overall Total Metal bound (mg)	†% reduction cadmium bound		‡% change in overall amount of metal bound
	Cd^{2+}	Cu^{2+}	Zn^{2+}			A	B	
<u><i>A.viscosus</i></u>	0.43	0.33	0.02	3.0	0.78	-38	28	+30
<u><i>A.globiformis</i></u>	0.13	0.80	0.01	3.0	0.94	46	73	+96
<u><i>K.aerogenes</i> 9128</u>	0.44	0.44	0.17	3.0	1.01	-18	47	+47

NB: * Expresses as mg of metal bound.

† versus, column (A) 10mg/l cadmium, and column (B), 30mg/l cadmium solution. Negative reduction indicates enhanced uptake.

‡ + = % increase in amount of metal bound vs 30mg/l cadmium
 - = % decrease in amount of metal bound vs 30mg/l cadmium

TABLE 15: *Biosorption of Metal Ions from 50mg/l Solutions of Five Different Metals containing 10mg/l of each Metal

ORGANISM	METAL ION					Overall Initial Metal total present (mg)	Overall Total Metal bound (mg)	l% Reduction Cd bound		x% Change in Overall amount of metal bound
	Cd ²⁺	Cu ²⁺	Pb ²⁺	Ni ²⁺	Zn ²⁺			A	B	
<u>A.viscosus</u>	0.30	0.27	0.86	0.00	0.00	5.0	1.43	3	65	+59
<u>K.aerogenes</u> 9128	0.18	0.42	0.43	0.19	0.15	5.0	1.37	50	83	+77

136

NB: pH 7 ± 0.5; 20°C.

* Expressed as mg of metal removed by 30mg of biomass

^l Reduction in amount of Cd²⁺ bound compared to amount of Cd²⁺ bound in 10 and 50mg/l solution of Cd²⁺ alone.
(Column A = 10mg/l; B = 50mg/l)

^x + = % increase in amount of metal bound compared to 50mg/l Cd²⁺
- = % decrease in amount of metal bound compared to 50mg/l Cd²⁺

presents these changes in a mixed solution containing 10mg/l each of Pb^{2+} , Cu^{2+} , Cd^{2+} , Ni^{2+} and Zn^{2+} for each of these strains and Table R17 illustrates the changes that occurred in an equimolar solution of Cd^{2+} and Ni^{2+} .

Overall, the total amount of metal bound in mixed metal solutions was greater than the amount of cadmium bound in a cadmium solution containing an equivalent amount of metal (with the exception of K.aerogenes and A.globiformis in a $\text{Cd}^{2+}/\text{Cu}^{2+}/\text{Zn}^{2+}$ solution containing 1mg/l of each metal ion).

It also emerged that a reduction in the amount of cadmium bound occurred in mixed metal solution, suggesting competition for binding sites between cadmium and other metals. As less cadmium could be bound by biomass when other metal ions were present, these metal ions (Pb^{2+} , Cu^{2+} , Ni^{2+} and Zn^{2+}) in combination would appear to bind to the same sites as cadmium ions to some degree. The overall amount of metal bound in mixed metal solutions was greater than the amount of cadmium bound from a solution of equivalent concentration of cadmium alone. Thus there is evidence that these metal ions do not all associate with the same binding sites.

Qualitative comparison of the levels of metal ions bound in mixed solution confirms that no specific uptake

TABLE 16: ***Biosorption of 0.5mg/l each of Cadmium and Nickel by 30mg *A.viscosus***

METAL IONS		Initial Total metal (mg)	Overall Total bound (mg)	% Reduction [⊕] Cadmium bound		[†] % Change in overall amount of metal bound
Cd ²⁺	Ni ²⁺			A	B	
0.038	0.0105	0.100	0.0485	10	52	+13

* Expressed as mg of metal bound: pH = 7 ± 0.5; 20°C

[⊕] Versus; Column A 0.5mg/l initial cadmium: Column B 1mg/l initial cadmium

[†] + = Increase in amount of overall metal uptake vs 1mg/l cadmium
 - = Decrease in amount of overall metal uptake versus 1mg/l cadmium

preference is maintained by the biomass types examined. At different overall concentrations of a $\text{Cd}^{2+}/\text{Cu}^{2+}/\text{Zn}^{2+}$ equimolar mixture the distinct affinity for copper observed in individual metal solutions was not maintained.

Several examples are known of metal ions competing for binding sites of biomass. Strandberg et al (1981) demonstrated displacement of uranium ions from S.cerevisiae by calcium ions. Cadmium uptake by Aureobasidium pullulans and S.cerevisiae was reduced in presence of calcium ions and iron II and manganese II ions affected zinc uptake by Neocosmospora vasinfecta (Gadd, 1986). Uranium binding by R.arrizhii was less in presence of Fe^{2+} and Zn^{2+} (Tsezos and Volesky, 1982). When the uptake of individual metals is considered, some specificity appears to exist as some metals are accumulated to a greater degree than others. With the species investigated Cu^{2+} ions had the highest affinity for the biosorbents. However, in mixed metal solution, competition does occur.

3.2.8 Effect of growth medium and culture age on cadmium biosorption

(i) Growth media

Many biological polymers may contain potential metal

binding sites (Hunt, 1986). For this reason when investigating the uptake of cadmium by biomass, no nutrients were included. The supposition that some growth nutrients might bind cadmium was investigated in a series of experiments . The results of these experiments are presented in Table 17 . Both nutrient broth (OXOID) and the glucose-rich growth medium usually employed for culture maintenance were capable of removing cadmium from solution.

In addition, it may be seen that A.viscosus biomass in presence of nutrient bound 14% more cadmium and 5% more cadmium at 21 and 41mg/l initial cadmium respectively, than A.viscosus biomass alone. Thus presence of some biochemicals does influence biosorption. (Note that all cadmium biosorption determinations carried out during this project with biomass were carried out after cells had been separated from the growth medium and washed.)

(ii) Culture age

Bacterial strains classified as in the genus Arthrobacter have a characteristic life cycle in which the cell morphology changes, as the cells appear as rods in young cultures and as cocci in old cultures or conditions of nutrient depletion (Keddie and Jones, 1983). This change in cell morphology was considered possibly to have some effect, so the binding capacity of

TABLE 17: Biosorption of Cadmium by Culture Medium

	UGM		ONB		<u>A.viscosus</u>		<u>A.globiformis</u>		<u>A.viscosus</u> + UGM		<u>A.viscosus</u> + ONB	
Amount of Cadmium bound (mg)	0.378	0.533	0.084	0.082	0.504	0.820	0.399	0.615	0.798	1.025	0.546	0.984
Dry matter present (mg)	14.4	14.4	10.4	10.4	37.0	37.0	30.0	30.0	42.0	42.0	45.0	45.0
% of Cadmium bound	18	13	4	2	24	20	19	15	38	25	26	24
Cadmium concentration (mg/l)	21	41	21	41	21	41	21	41	21	41	21	41

N.B.:

UGM = Usual growth medium, comprising 10g/l D-Glucose

ONB = OXOID Nutrient Broth

FIG.32 EFFECT OF CULTURE AGE ON CADMIUM UPTAKE
FROM 10mg/L SOLUTION BY 30mg A.VISCOSUS

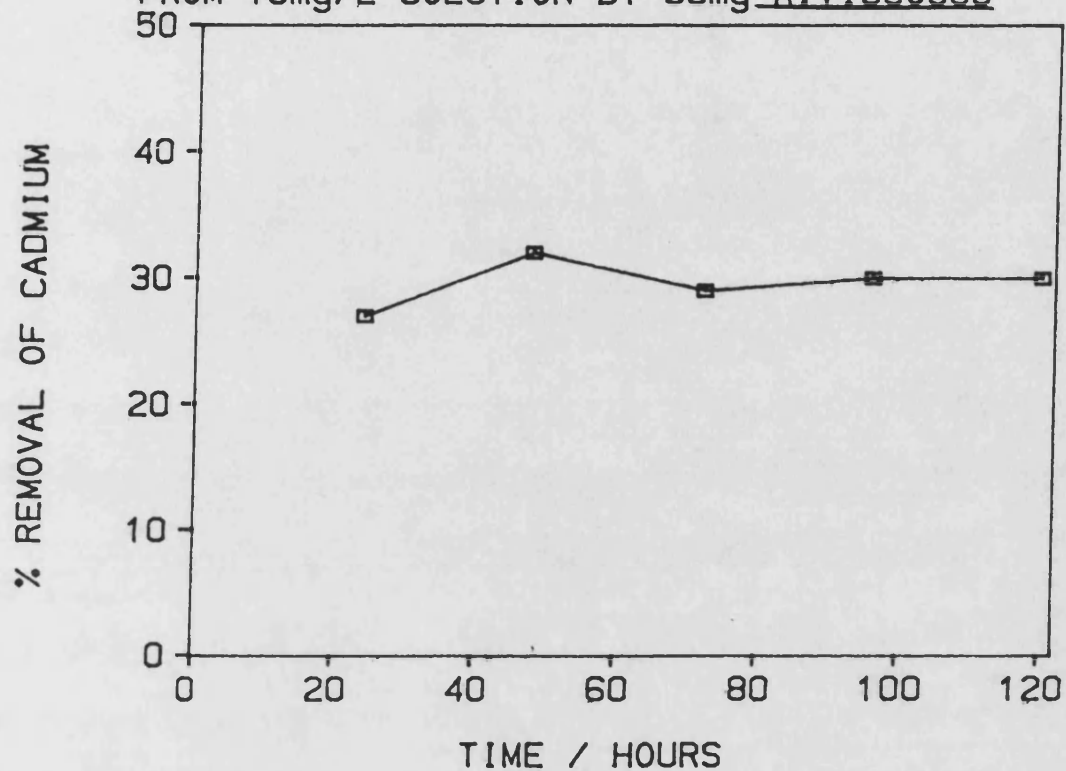
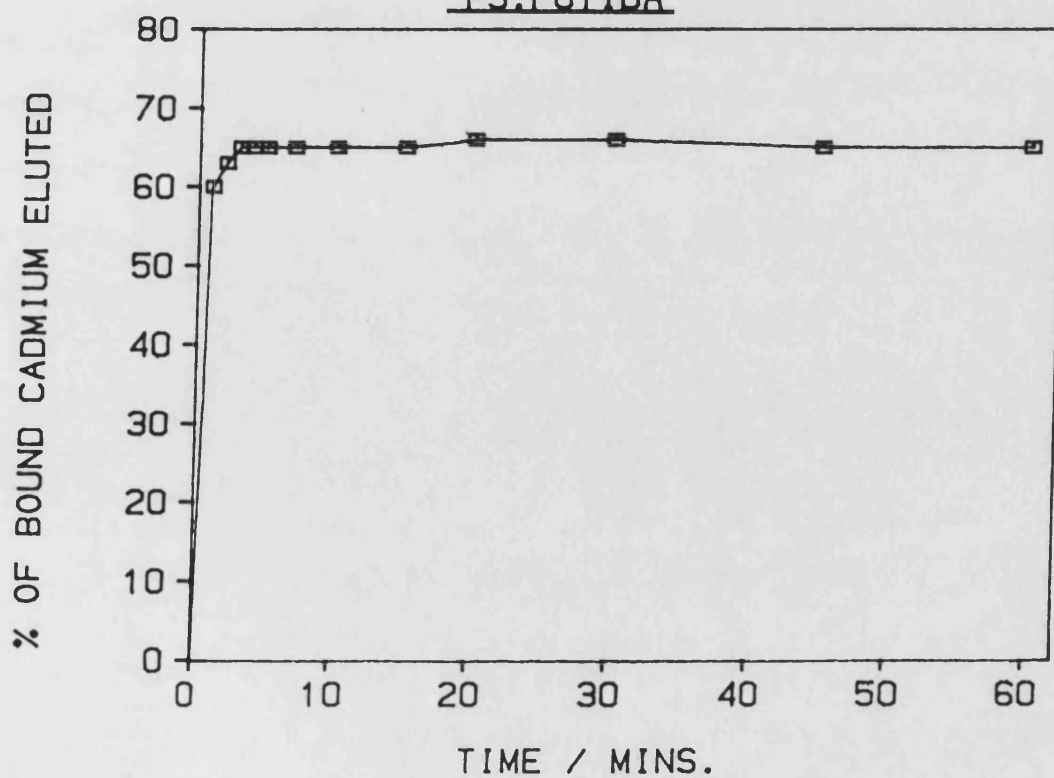


FIG.33 KINETICS OF CADMIUM DESORPTION FROM 30mg
PS.PUTIDA



equivalent biomasses of A.viscosus 9728 was determined from 24 up to 120 hours after inoculation from exponential growth phase culture. Gram stained slides of the culture were taken whenever a biomass sample was prepared for cadmium biosorption. At 24 hours the culture was predominantly (i.e. 78%) composed of rods with very few cocci; at 48 hours the culture was approximately 50% cocci and 50% rod-like cells. At 72 hours the culture was predominantly cocci with very few rods present. At 96 and 120 hours the culture was virtually totally composed of cocci.

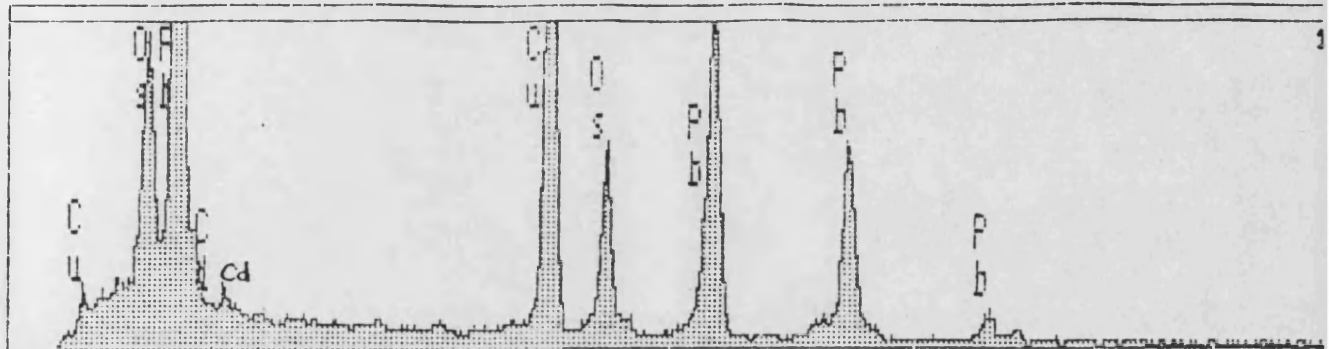
The fluctuations occurring in cadmium binding capacity were minor throughout the period from 24 to 120 hours and did not produce significant changes in biosorptive capacity of any major significance. Thus the change in cellular morphology associated with the growth cycle of A.viscosus and the biochemical changes involved with it (Ensign and Wolfe, 1964) did not significantly alter the cadmium binding capacity of the biomass. (The biomass recovered from 24 hour culture was least effective at cadmium binding; possibly because polysaccharide synthesis was greater after 24 hours culture.)

3.2.9 Analysis of cadmium exposed biomass by Transmission Electron Microscopy and X-ray spectroscopy

Samples of biomass (30mg) were exposed to 1000mg/l cadmium for five minutes then recovered and prepared for transmission electron microscopy and x-ray spectroscopy. The observations recorded for each bacterial species will now be dealt with each in turn.

(1) A.viscosus

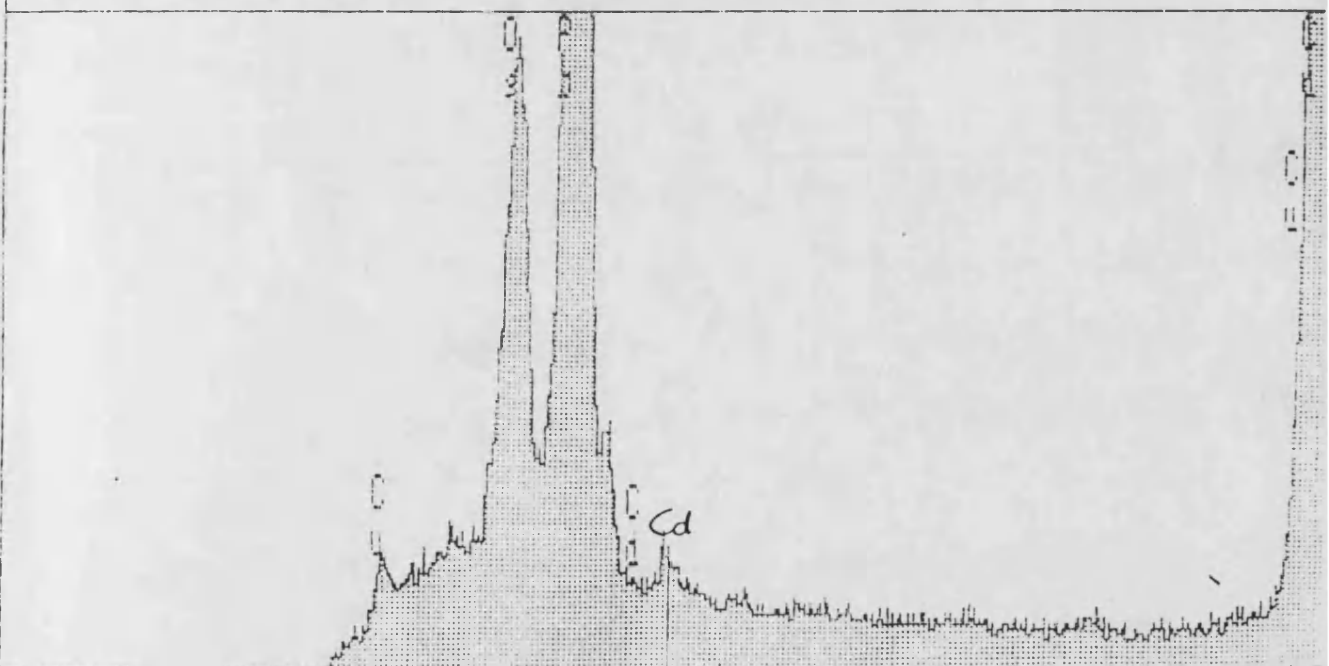
Electron microscopy revealed that in stained and unstained samples dark (i.e. electron dense) inclusions were present within the cell cytoplasm. Usually more than one such dark region was present within the cells (Micrograph1a; Spectrogram 1) and x-ray examination of the cell cytoplasm and focussing on these dark inclusions showed that they were cadmium associated . Micrographs 1b and 2 are unstained preparations which again show dark inclusions. Spectrogram2 focussed on an area of cytoplasm containing no dark inclusions. Spectrogram 3 focussed on a dark inclusion (Micrograph 3) illustrates that a more prominent cadmium peak is obtained in the vicinity of the dark inclusion. Several such comparisons were made and it was apparent that high cadmium peaks were associated with high phosphorus peaks for A.viscosus. Compared to the localised accumulation of cadmium within the cell, less appeared to be associated with the cell wall. In addition, both rods and cocci were examined and no apparent difference in main site of cadmium binding was observed.



X-RAY

Live: 100s Preset: 100s Remaining: 0s

Real: 128s 22% Dead

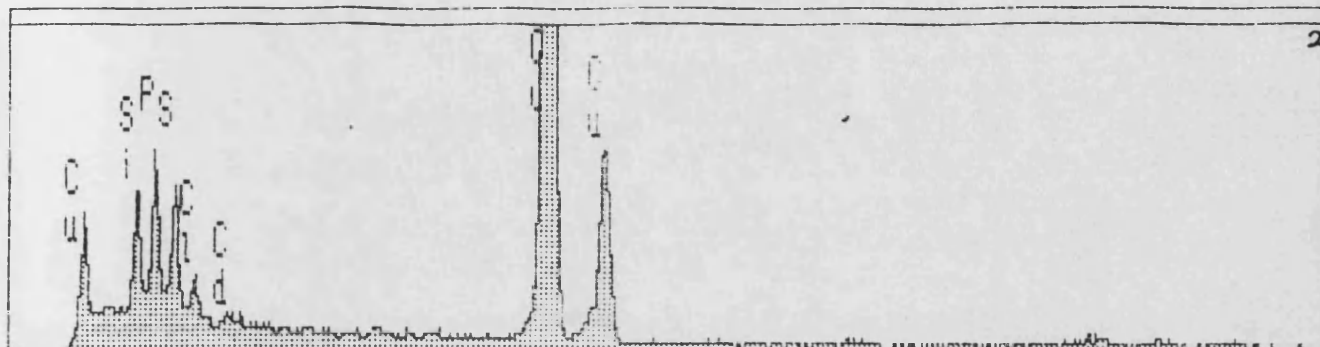
FS= 1K
MEM1:

3.140 keV

ch 167=

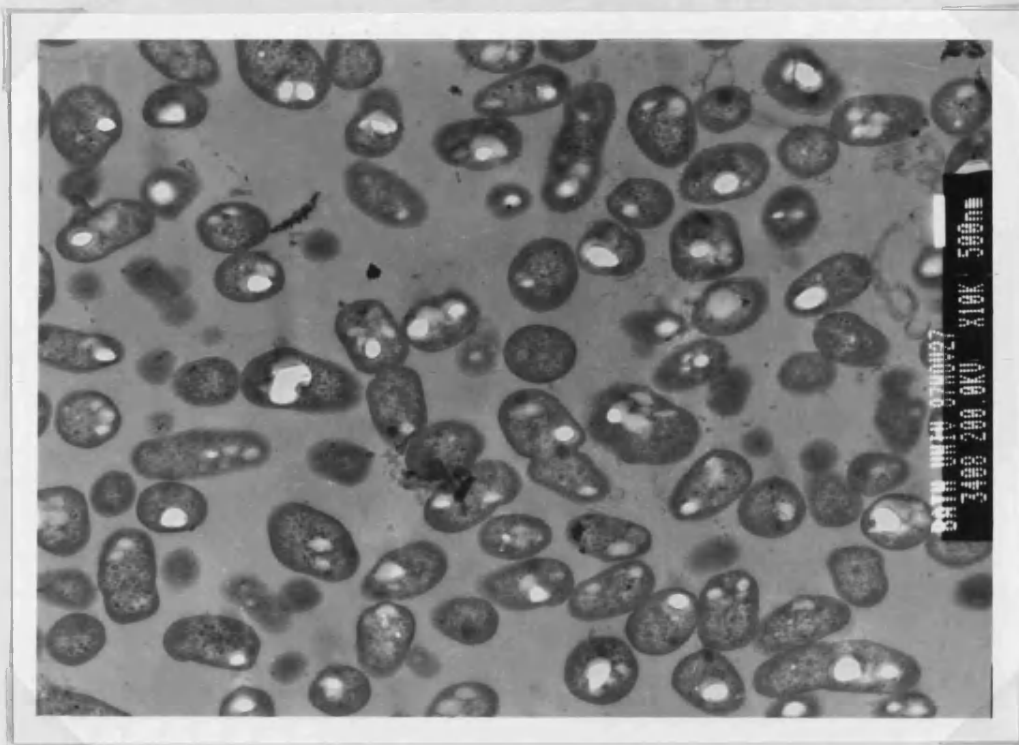
8.3 >

195 cts

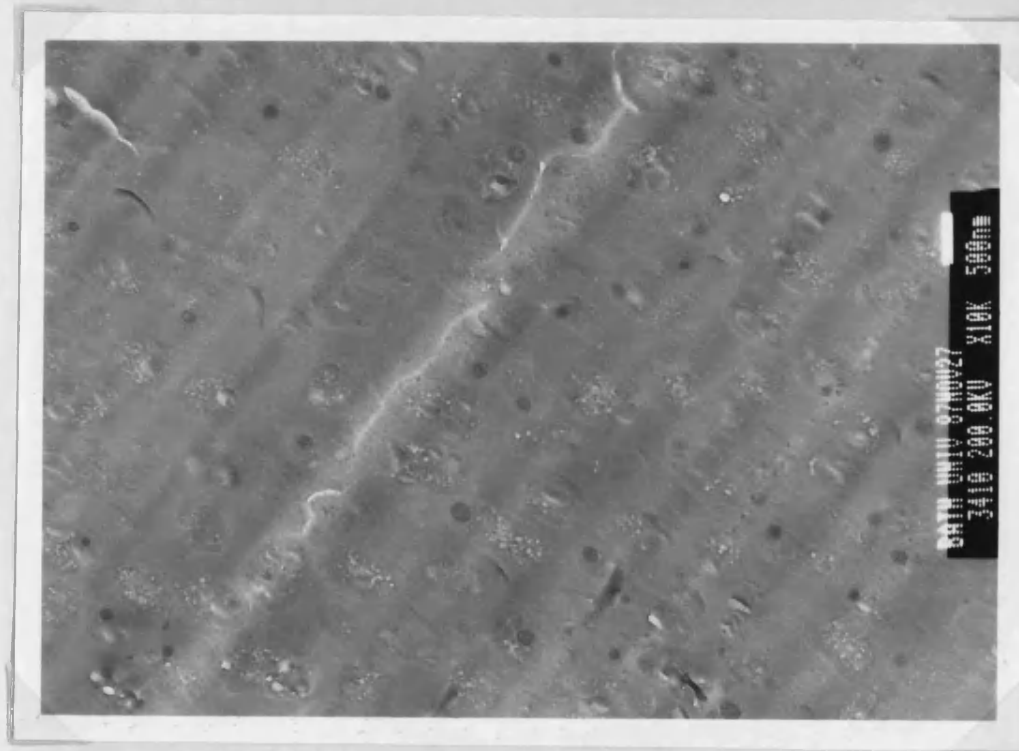


X-RAY

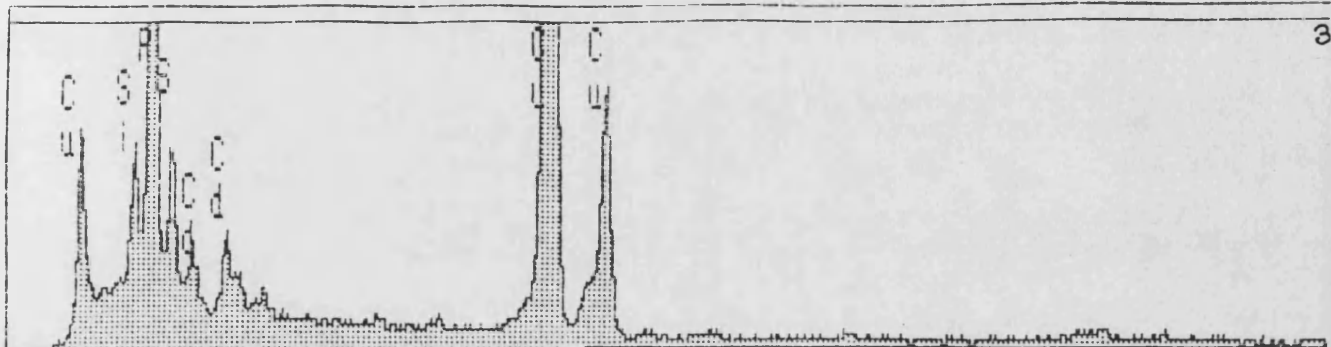
```
< .9          3.440    keV          6.0 >
FS=    1K          ch    182=          76    cts
MEM1:
```



Micrograph 1a

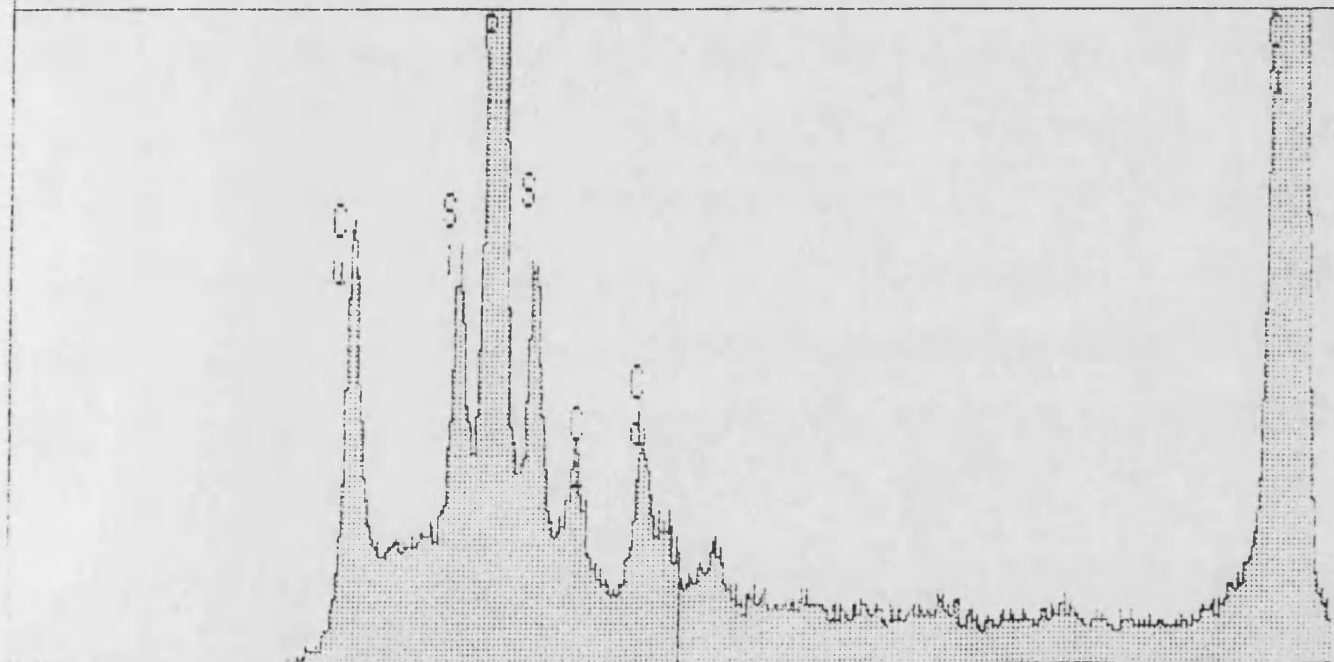


Micrograph 1b

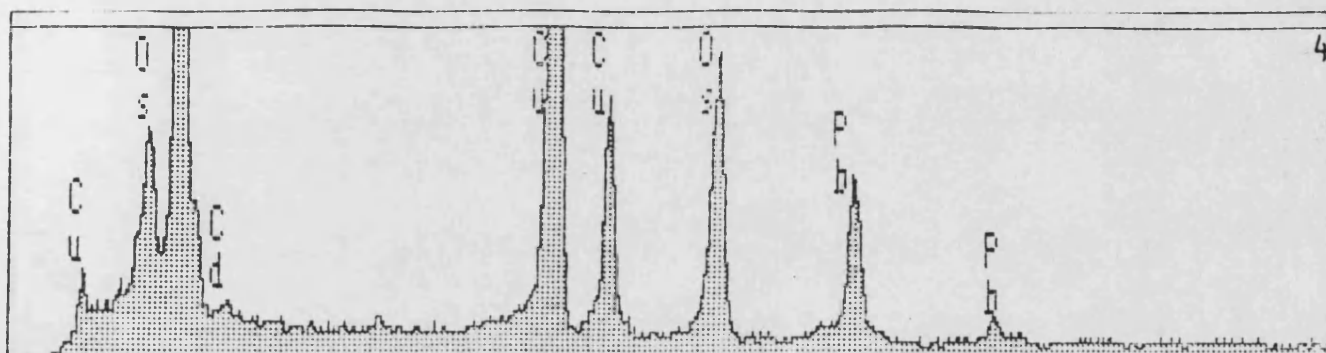


X-RAY

Live: 100s Preset: 100s Remaining: 0s
 Real: 133s 25% Dead

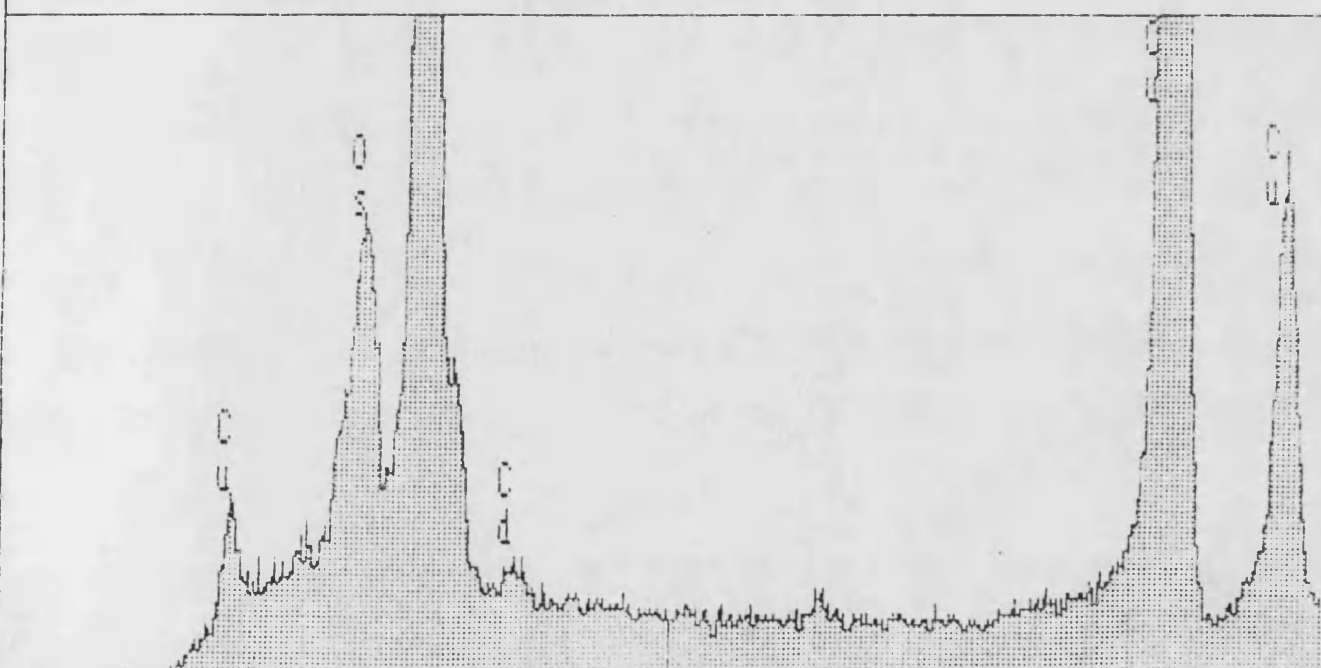


FS= 1K 3.440 keV 8.6 >
 MEM1: ch 182= 183 cts



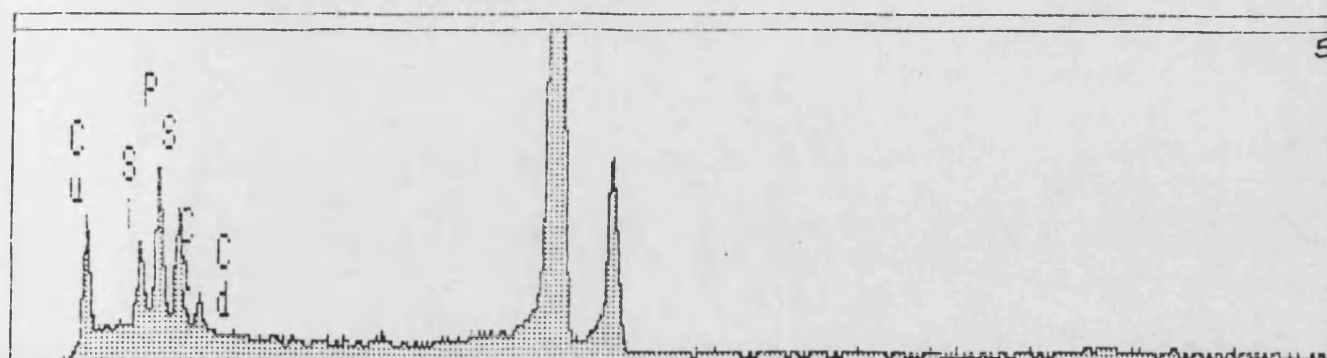
X-RAY

Live: 100s Preset: 100s Remaining: 0s
 Real: 155s 35% Dead



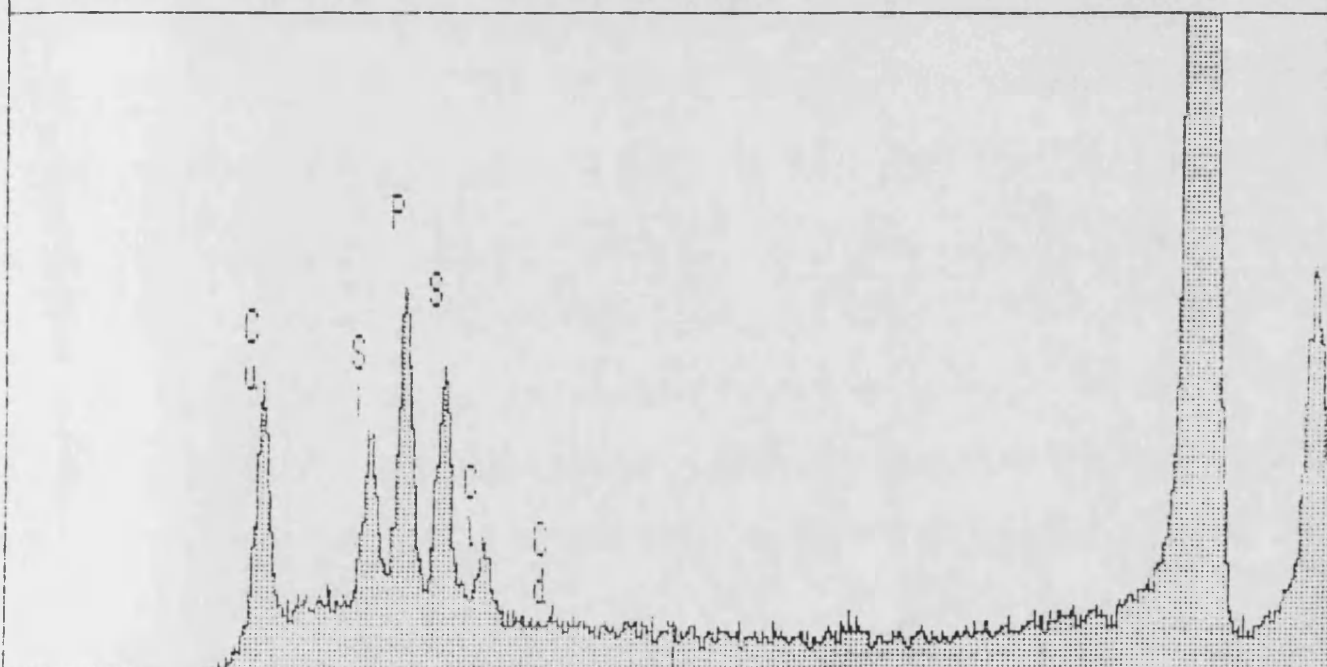
FS= 1K
 MEM1:

4.300 keV 9.4 >
 ch 225= 88 cts

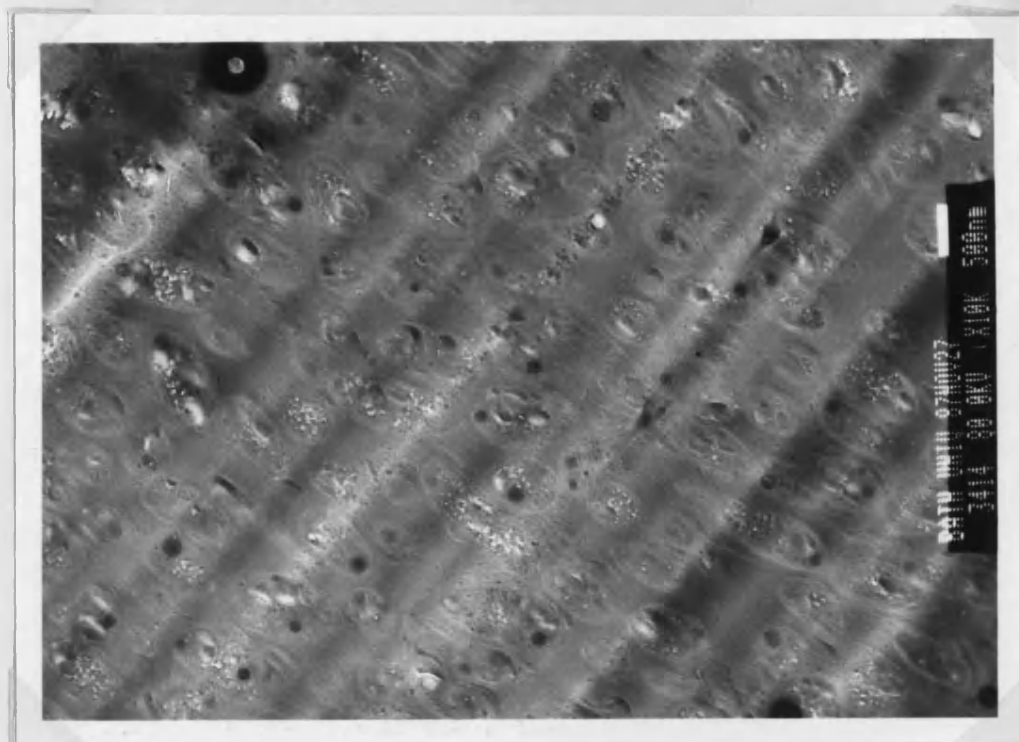


X-RAY

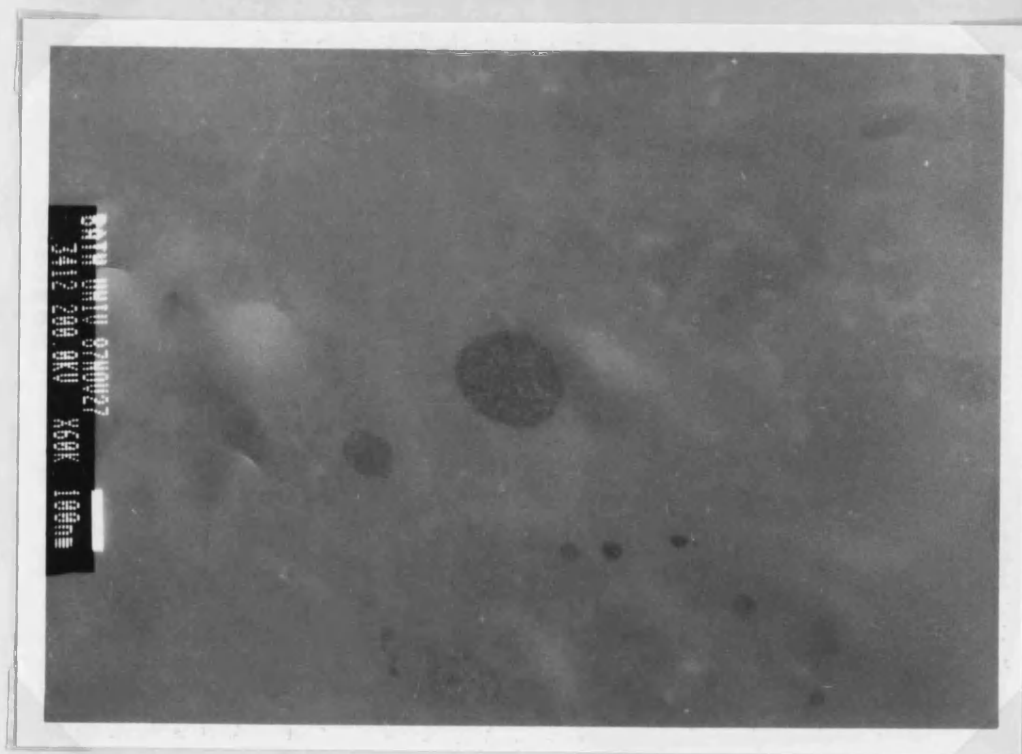
Live: 100s Preset: 100s Remaining: 0s
Real: 137s 27% Dead



FS= 1K 4.100 keV 9.2 >
MEM1: ch 215= 81 cts



Micrograph 2



Micrograph 3

(2) A.globiformis

The cadmium peak associated with cell cytoplasm (Spectrogram 4) was lower than that for A.viscosus and indeed no obvious localised sites stained more heavily than others were found in unstained samples, or stained samples (Micrograph4 - stained). Outside the cell no cadmium peak appeared on spectrograms (Spectrogram 5).

(3) P.putida

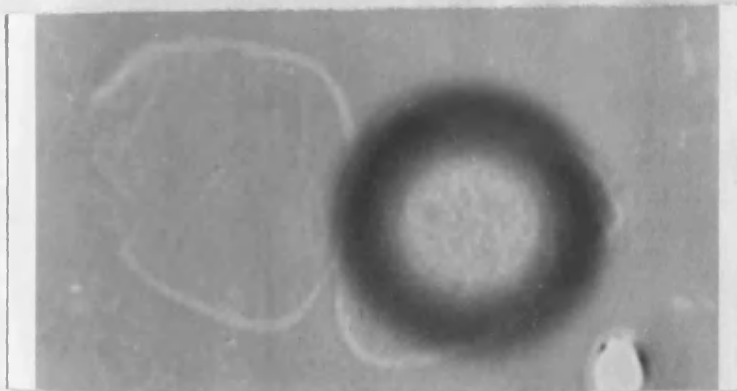
Localised electron-dense (i.e. dark) inclusions were present within P.putida cells (Micrograph 5). These inclusions were associated with cadmium (Spectrogram 6) and were more numerous and not as large as those present within A.viscosus cells. No particular element appeared to be significantly associated with these inclusions.

(4) P.cruciviae

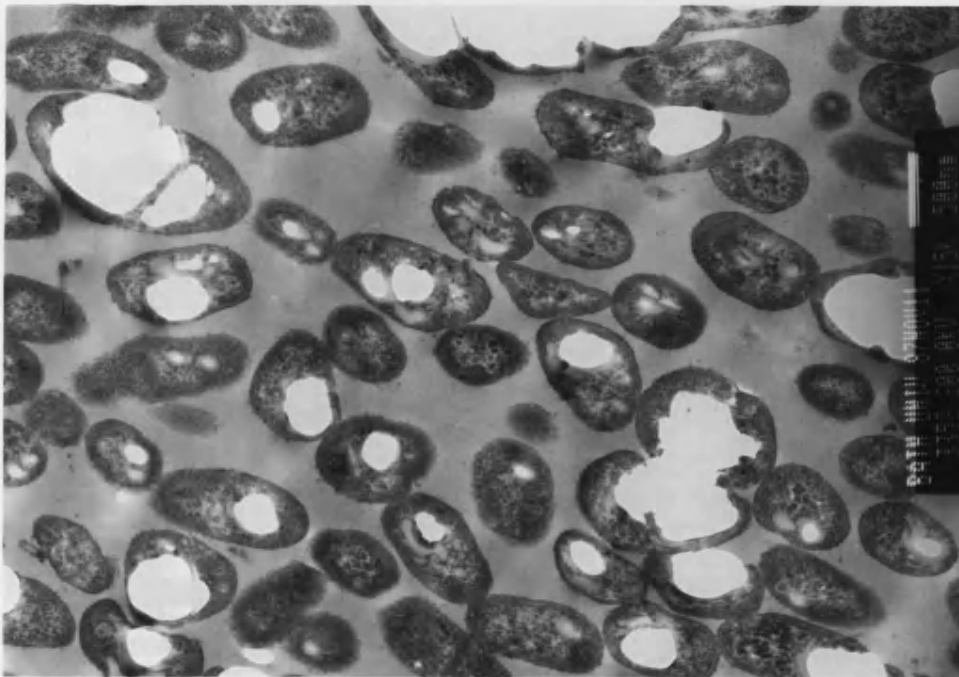
Spectrogram 7 illustrates the cadmium content of two cells (Micrograph 6- unstained) . Focussing on the cell interior revealed that cadmium was associated with the cytoplasm (Spectrogram8). However no obvious localised accumulations of cadmium were present such as those identified with P.putida cells.

(5) K.aerogenes strains 9527and 9128

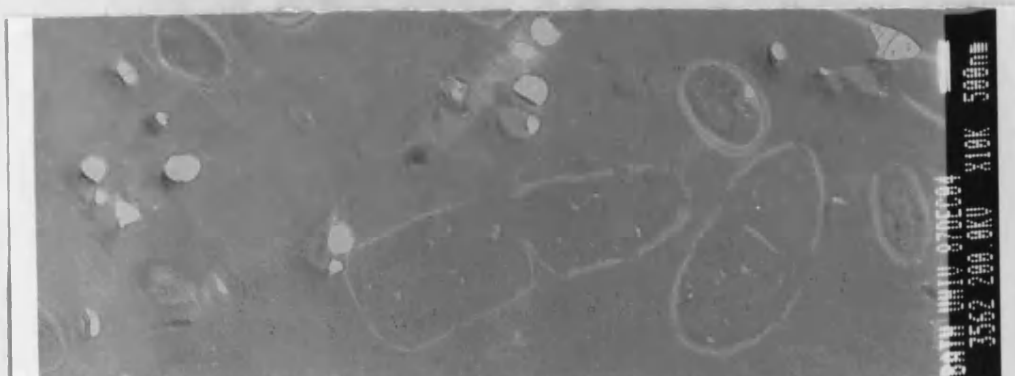
Spectrogram 9 was focussed on the cell boundary of a



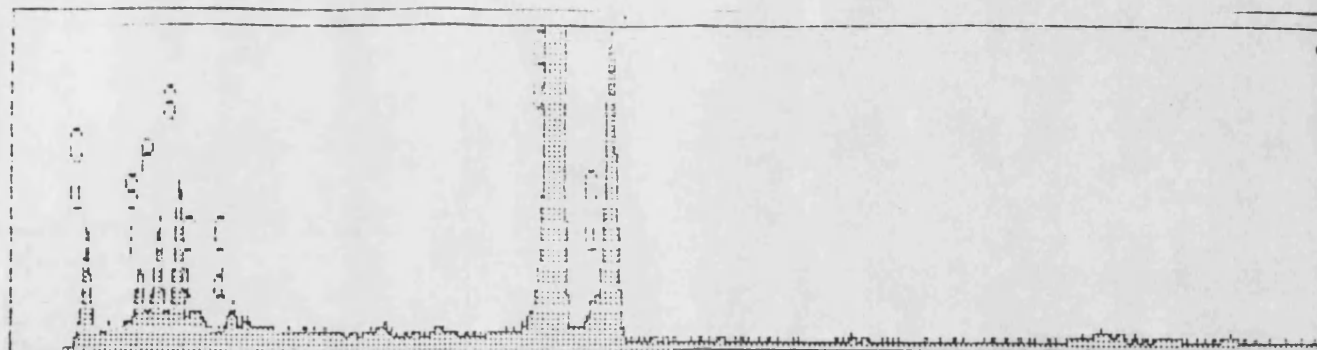
Micrograph 4



Micrograph 5

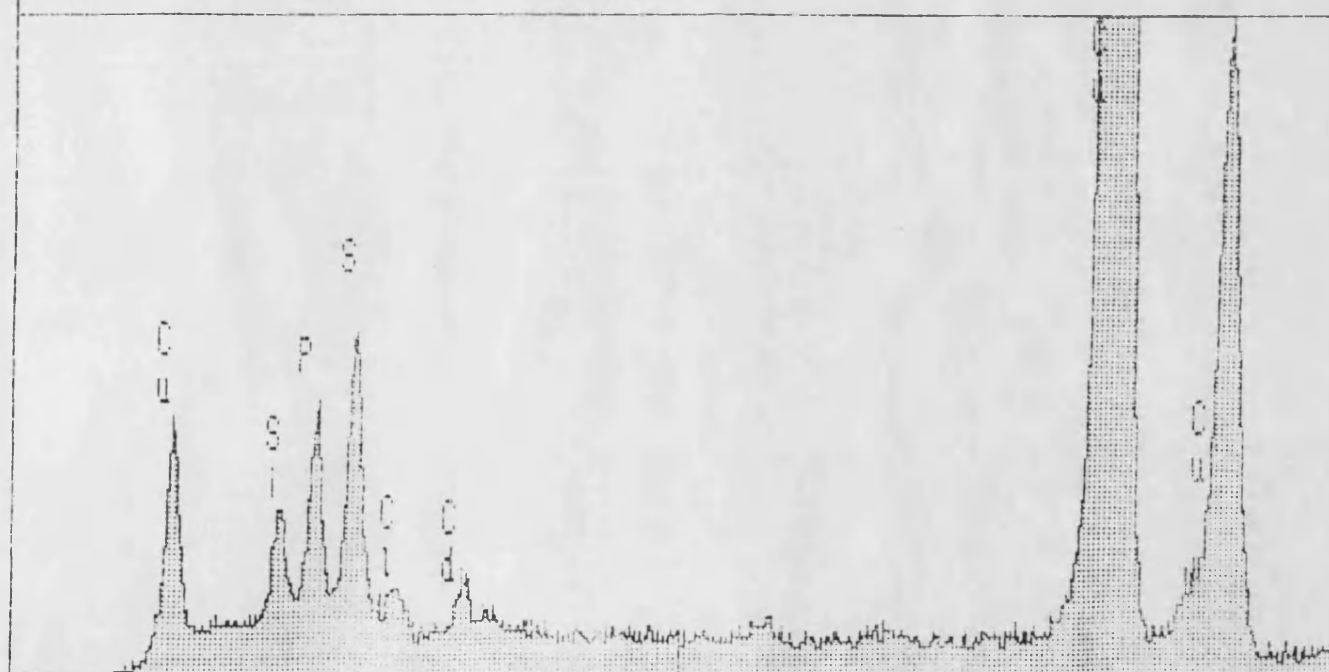


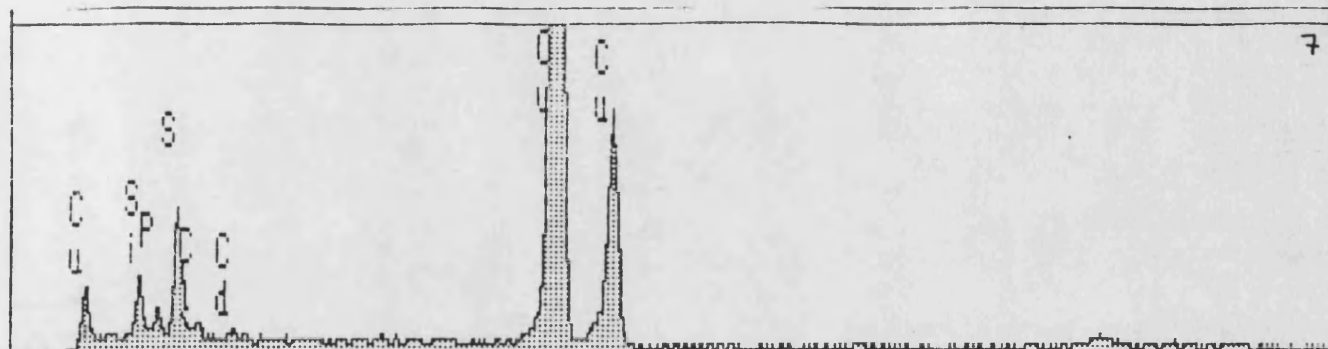
Micrograph 6



X-RAY

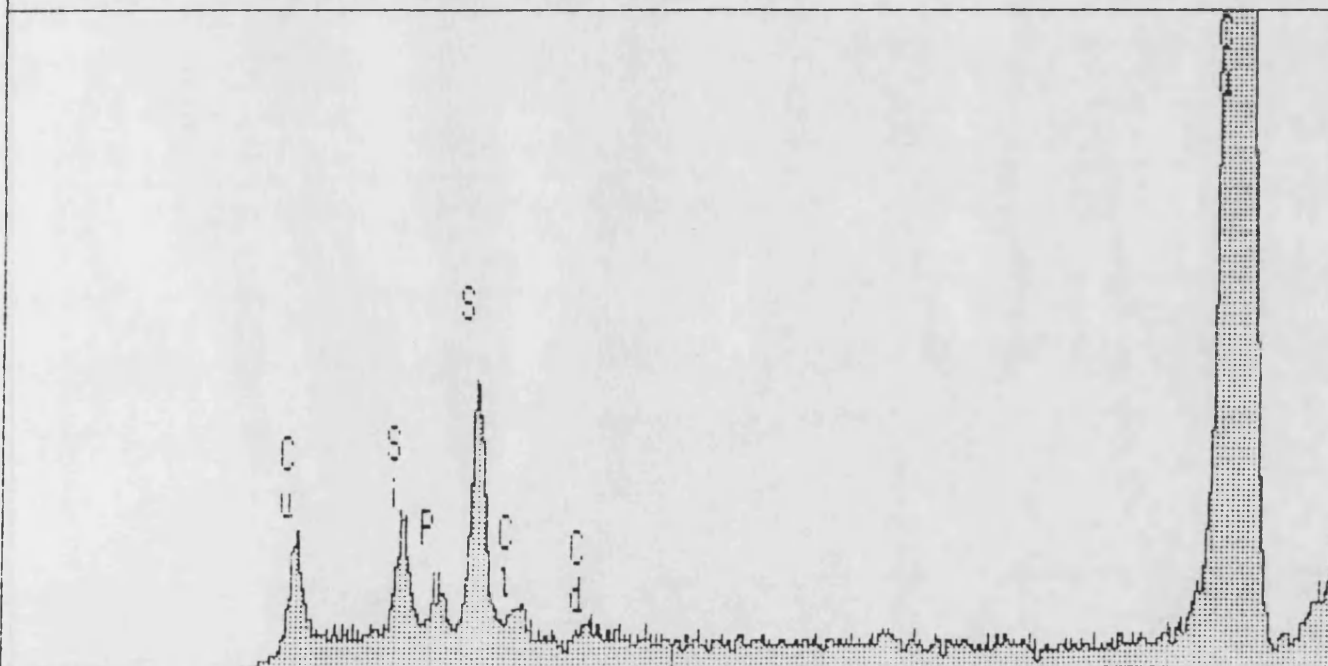
Live: 100s Preset: 100s Remaining: 0s
Real: 155s 35% Dead





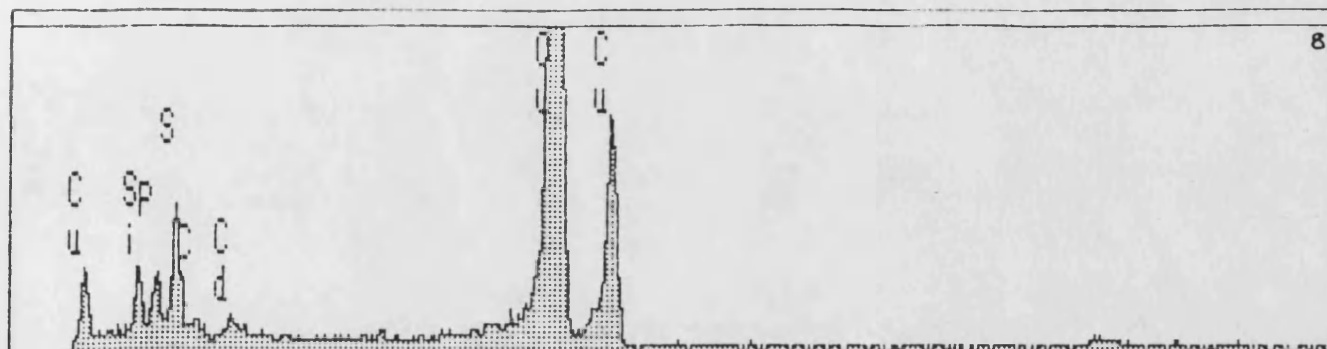
X-RAY

Live: 100s Preset: 100s Remaining: 0s
 Real: 130s 23% Dead



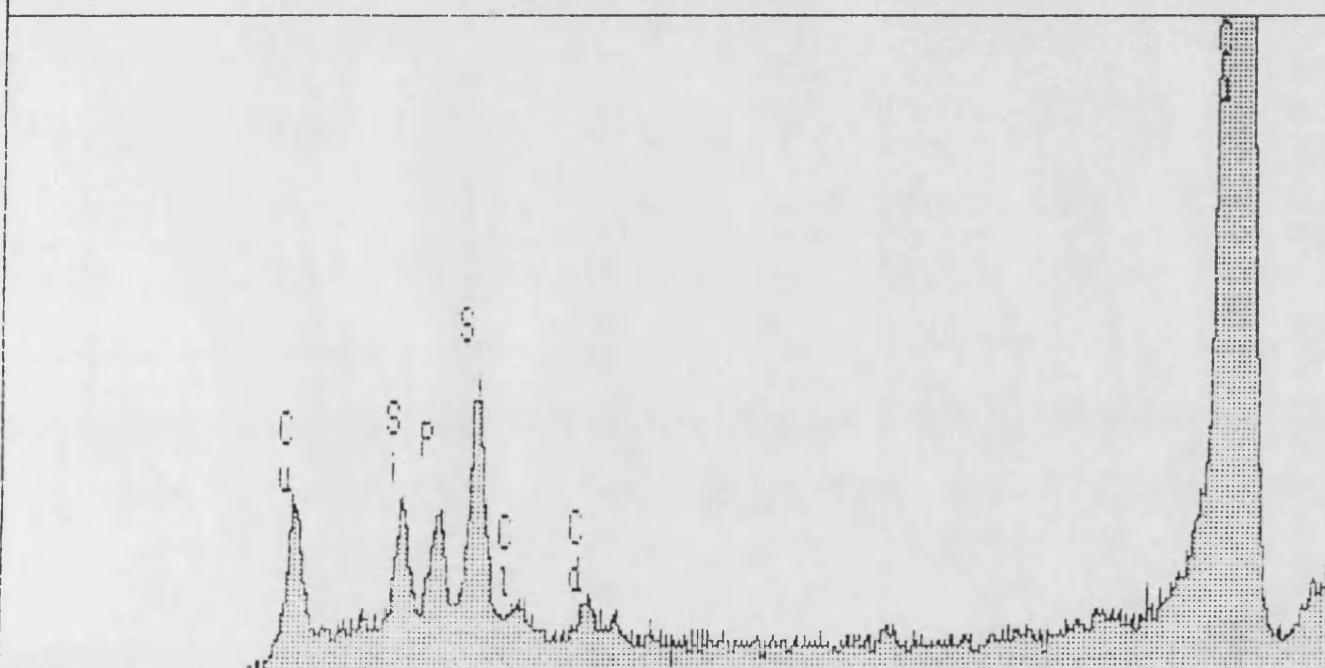
FS= 1K
 MEM1:

3.820 keU 8.9 >
 ch 201= 45 cts



X-RAY

Live: 100s Preset: 100s Remaining: 0s
 Real: 133s 25% Dead



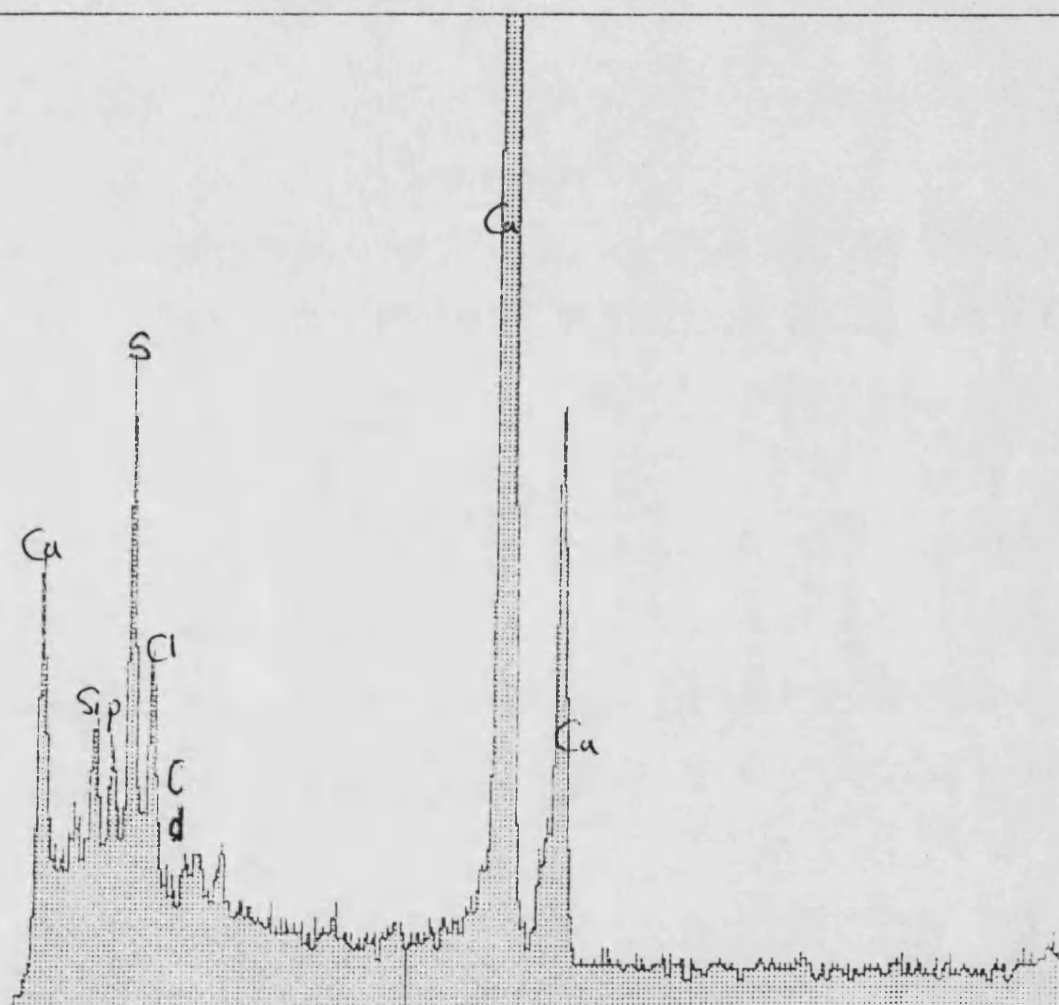
FS= 1K
 MEM1:

3.820 keV 8.9 >
 ch 201= 70 cts

X-RAY

9

Live: 100s Preset: 100s Remaining: 0s
Real: 148s 32% Dead



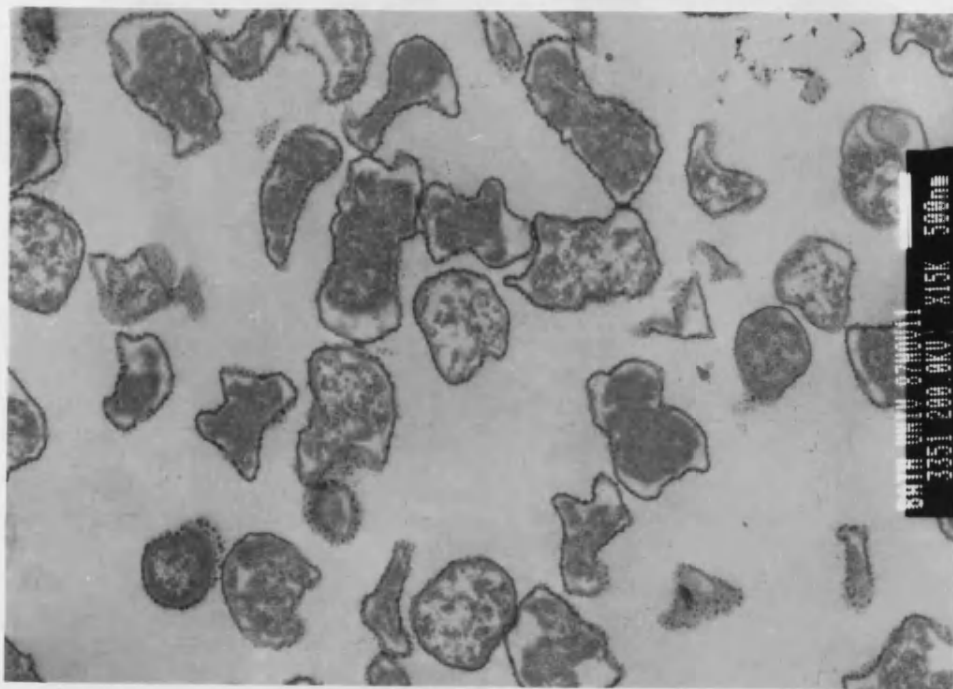
FS= 1K
MEM1:

6.540 keV

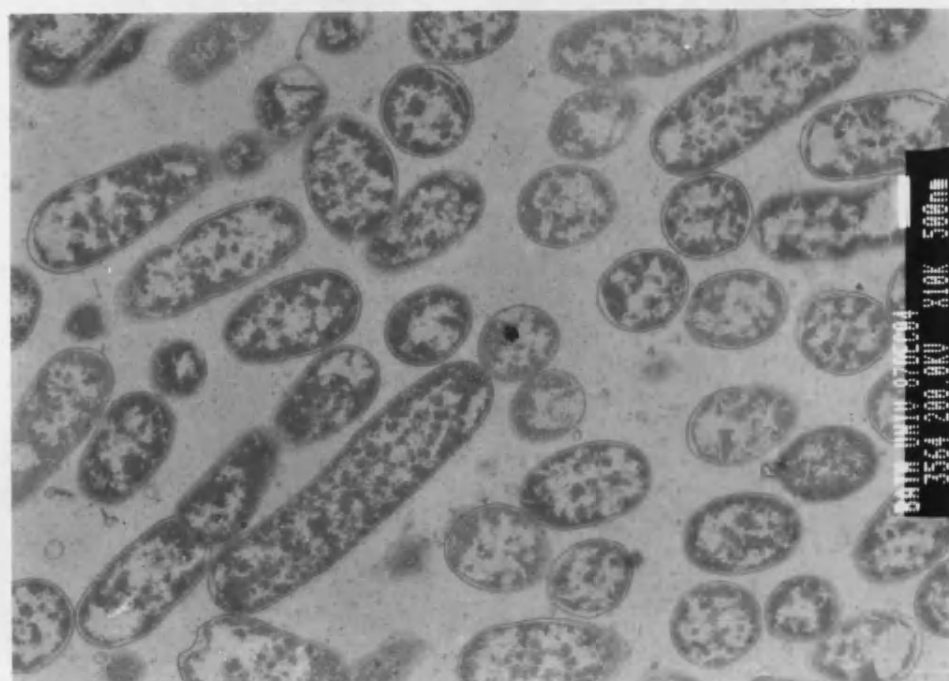
ch 337=

16.8 >

70 cts



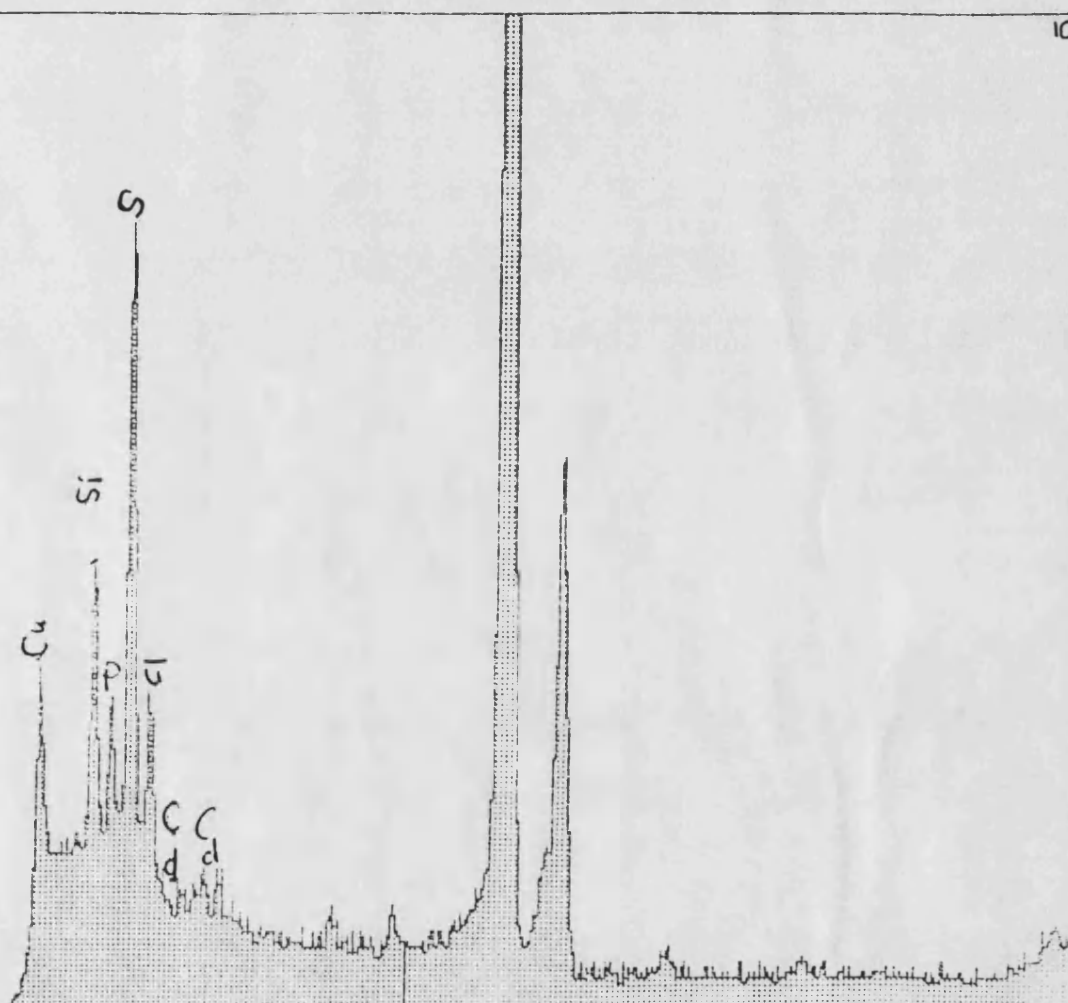
Micrograph 7



Micrograph 8

X-RAY

Live: 100s Preset: 100s Remaining: 0s
Real: 126s 21% Dead



FS= 1K
MEM1:

6.540 keV 16.8 >
ch 337= 75 cts

X-RAY

Live:

100s

Preset:

100s

Remaining:

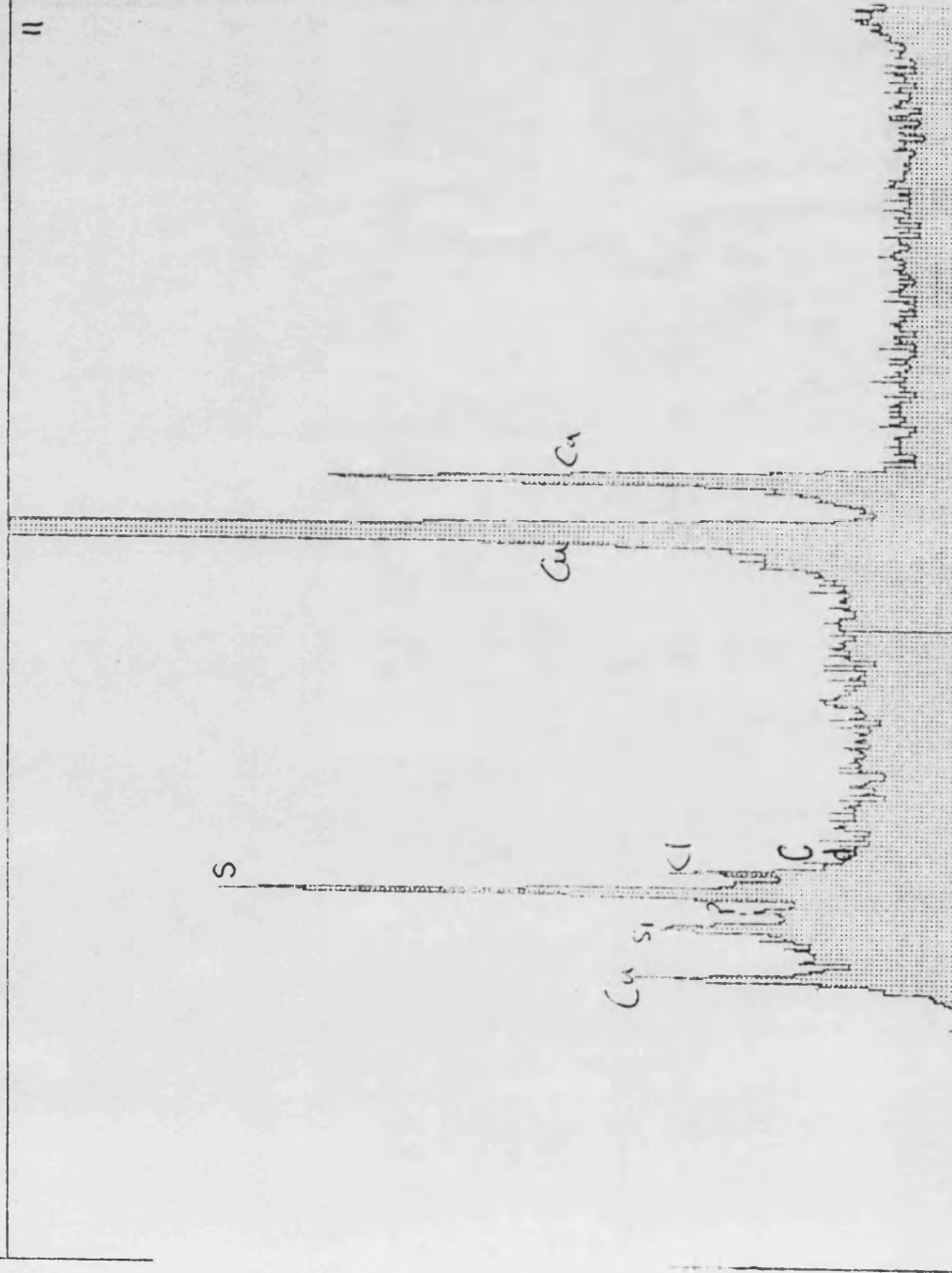
0s

Real:

138s

28%

Dead

FS=511
MEM1:

6.540 keV

ch 337=

16.8 >

64 cts

K.aerogenes 9527 sample cell. For both strains 9527 and 9128 some cadmium was associated with the cell interior (Spectrogram 9), but some appeared associated with the boundary of the cell (Spectrogram 10). Micrograph 7 shows presence of a darker staining area outside the cell wall of K.aerogenes 9128 cells (arrowed). It should be stressed that the X-ray beam itself could not be focussed on the cell wall to the exclusion of cytoplasm and some part of the cell interior. For both strains cadmium appeared to be associated with presence of sulphur.

(6) K.aerogenes 9528

As with the two other K.aerogenes strains, no obvious localised deposits were present within or around the boundary of the cell (Micrograph 8). Unstained and stained samples showed little cadmium (Spectrogram 11), but where cadmium was present it appeared to be associated with a sulphur peak (Spectrogram 11; unstained sample).

Apart from the presence of cadmium, stained samples contained lead (Pb) and osmium (Os). The samples were mounted on a copper grid and cut with a glass knife which is responsible for the peaks of silicon (Si) and copper (Cu).

These observations suggest that A.viscosus,

A.globiformis, P.cruciviae and P.putida bind cadmium intracellularly. Intracellular metal binding by a pseudomonad has already been demonstrated with uranium (Strandberg et al, 1981). Cadmium accumulation by A.viscosus was localised and phosphorus associated which might indicate presence of a cadmium detoxification system involving production or accumulation of a phosphorus containing compound. Alternatively, cadmium might be bound within the cell to structures rich in phosphorus; examples being nucleic acids or the phosphomannans of S.cerevisiae (Strandberg et al, 1981). The former explanation is most likely in view of the amount of phosphorus detected in association with cadmium and the localised accumulation observed within the bacterial cell. Aiking et al (1984) demonstrated presence of a cadmium detoxification system in K.aerogenes that involved accumulation of phosphate. A similar system would appear to operate in A.viscosus with localised accumulation of precipitated cadmium within the cell. The higher cadmium biosorption capacity of A.viscosus over A.globiformis, and also its better survival on exposure to cadmium, may be attributed to this mechanism as exopolysaccharide production has been demonstrated (see previous) to account for only part of the difference in biosorptive capacity of the two Arthrobacter species.

P.putida proved a better biosorbent than P.cruciviae. Electron microscopy showed the presence of localised dark inclusions which were cadmium associated within P.putida, but no localised cadmium accumulation occurred within P.cruciviae, although the latter did have cadmium within the cell.

Higham et al (1985) found evidence of cadmium resistance mechanisms within P.putida. At low concentrations of cadmium some efflux of the ion from the cell was apparent, but the cells were also capable of growth in presence of high intracellular cadmium accumulation. The localised accumulation identified in this investigation is due to the latter mechanism for P.putida and is probably responsible for it having a higher biosorption capacity than P.cruciviae.

Cadmium binding by the K.aerogenes strains was not predominantly intracellular under the conditions employed. The cell membrane and wall could not be isolated by the analysis technique used, but electron microscopy did not suggest any darker staining within this region that might be cadmium associated. Accumulation of metal ions by the extracellular polysaccharide produced by K.aerogenes is known to occur (Bilton and Freihofer, 1978; Brown and Lester, 1982). It is possible that as little as no cadmium could be detected within cells of K.aerogenes 9128 and 9527, which produced extracellular polysaccharide, that it was associated with the polysaccharide capsule. The capsule may have been reduced or lost during preparation of the samples for electron microscopy. Micrograph 7 shows presence of a layer surrounding a K.aerogenes 9128 cell which might be a reduced polysaccharide layer. It

should be noted that light microscope examination of the culture used showed presence of a capsule.

This hypothesis gives no explanation of why K.aerogenes 9528, the non-capsule former, also showed little obvious intracellular cadmium accumulation. This does not necessarily invalidate the hypothesis for the following reasons:

(i) K.aerogenes 9528 was capable of exopolysaccharide production but did not retain it in close association with the cell. As with A.viscosus, the polysaccharide was excreted into the medium, and itself may have removed cadmium.

(ii) Aiking et al (1982, 1984) have carefully investigated cadmium uptake by K.aerogenes strains under continuous culture and discovered two resistance mechanisms under conditions of low glucose to preclude polysaccharide formation. A sulphide producing mechanism which gave localised accumulation of cadmium around the cell was noted (Aiking et al, 1982) as well as a system connected with phosphate accumulation which resulted in diffuse intracellular cadmium accumulation (Aiking et al, 1984). Localised precipitates of this nature were not present around any of the K.aerogenes cells in electron micrographs. This, plus the inducible nature of the sulphide detoxification mechanism (Aiking et al, 1982) would appear to rule out

this mechanism totally were it not for the moderate sulphur peaks detected by x-ray spectrography of all the K.aerogenes cells. These were not as extreme as the phosphorous peaks associated with A.viscosus cell interiors which leads to the following speculation. The presence of cadmium over the 10 minute exposure period may have induced the beginning of accumulation of sulphide to exclude cadmium from the cells. The sulphide producing mechanism excluded cadmium from the cell interior (Aiking et al, 1984) which would also implicate it in cadmium biosorption by K.aerogenes in this case. Absence of extreme levels of phosphorus, and of cadmium in the K.aerogenes cell interior rules out the phosphate accumulation in this case. Under the experimental conditions employed it would appear that the sulphide detoxification mechanism had been induced in all the K.aerogenes strains and it might be partially responsible for cadmium removal from solution. However, the electron micrographs suggest that the greater part of cadmium bound was in fact associated with external polysaccharide for strains 9128 and 9527 .

The mechanism and site of cadmium binding are important as they can dictate the potential of a bacterial (or fungal or other) species as a biosorbent. Intracellular accumulation of cadmium would make a biosorbent unsuitable if it resulted in low recovery, or increased

the treatment required for recovery of cadmium from the biomass. Thus the amount of cadmium that may be eluted from the cells by simple chemical treatment is of particular importance where intracellular uptake of metal is demonstrated.

3.3.0 Desorption

3.3.1 Desorption agents

For microbial biomass to be employed successfully in a metal recovery process, the metal bound to the biomass needs to be recovered easily (Brierly et al, 1986; Tsezos, 1984). Lowering pH is known to cause desorption of metal ions from biomass (Tsezos, 1984; Strandberg et al, 1981; Norberg and Persson, 1983) and other chemical agents have been found to achieve desorption of cell associated metal; particularly sodium carbonate or bicarbonate (Tsezos, 1984; Nakajuma et al, 1982).

Cadmium loaded cells of A.viscosus 9728 and P.putida were exposed to several potential chemical desorption agents. These included three mineral acids (sulphuric, nitric and hydrochloric) and two bases (sodium hydrogen carbonate and sodium carbonate). In addition Ethylene diamine tetraacetic acid (EDTA), Citric acid and sodium acetate were used with A.viscosus. The percentage desorption achieved was defined as the percentage of

cadmium bound by the biomass from a single exposure to cadmium which was subsequently released into solution following chemical treatment. Thus, where biomass was cadmium loaded, chemically treated and then re-loaded with cadmium, it is possible that over 100% desorption might be achieved on the second desorption sequence if all cadmium present were not desorbed during the first desorption treatment. The amount of cadmium eluted back into solution was determined after 10 minutes exposure to a desorption agent.

Low cadmium concentrations were employed to avoid occupying all biosorption sites during the first adsorption/desorption sequence. To consider the efficiency and usefulness of the chemical desorbents employed, two criteria were considered:

- (i) A large proportion of the cadmium attached to the cell should be eluted back into solution
- (ii) The cellular material should sustain as little damage as possible which might affect its biosorptive capability.

Of the mineral acids used to elute cadmium from A.viscosus biomass, hydrochloric acid proved the most efficient. Sulphuric acid was marginally more effective than hydrochloric acid at desorption during the initial adsorption/desorption sequence, but subsequent cadmium

TABLE 18: Desorption of Cadmium from 30mg *A.viscosus* by Different Potential Chemical Desorption Agents*

DESORPTION AGENT	<u>1st Sequence</u>			<u>2nd Sequence</u>		
	Cadmium bound (mg)	Cadmium desorbed (mg)	% Desorption	Cadmium bound (mg)	Cadmium desorbed (mg)	% Desorption
0.1M Sulphuric acid	0.0110	0.0101	92	0.0020	0.0000	0
0.1M Nitric acid	0.0110	0.0082	75	0.0022	0.0010	22
0.1M Hydrochloric acid	0.0100	0.0090	90	0.0032	0.0010	32
0.1M Citric acid	0.0100	0.0100	100	0.0100	0.0040	40
0.1M Sodium hydrogen carbonate	0.0110	0.0016	15	0.0230	0.0010	4
0.1M Sodium carbonate	0.0120	0.0004	3	0.0240	0.0000	0
0.1M Sodium acetate	0.0110	0.0000	0	0.0100	0.0020	20
0.1M EDTA	0.0120	0.0010	8	0.0150	0.0000	0

NB*: 15ml of 1mg/l cadmium solution in adsorption phase.

15ml of desorption agent added to recovered biomass for desorption phase.

biosorption with the same biomass was more efficient with hydrochloric acid. Examination of the amount of cadmium bound by the mineral acids during the second biosorption sequence shows that more cadmium was bound by hydrochloric acid treated A.viscosus biomass. This might indicate that hydrochloric acid was a gentler desorption agent than sulphuric or nitric acids, which correlates with the study of use of desorption agents with Rhizopus arrhizius by Tsezos (1984). Tsezos prepared samples of R.arrhizius biomass for electron microscopy following exposure to his desorption agents and determined that visible damage to the cell wall and interior was worst with sulphuric acid. Nitric acid was worse than hydrochloric acid, which Tsezos attributed to the stronger oxidising potential of nitric acid. Although no electron micrographs were prepared for biomass samples desorbed in this study, subsequent cadmium biosorption would suggest the same trend. This is reinforced when the effectiveness of citric acid, a much milder acid, is considered. Citric acid seemed to do no significant damage to the cadmium binding sites of A.viscosus as the same amount of cadmium was bound on the second adsorption/desorption sequence. However, subsequent desorption was reduced in each of the three repeated trials (each set of data in Tables 17 and 18 represents averages from triplicate trials). As citric acid is a mild acid it would not normally be expected to

TABLE 19: Desorption of Cadmium from 50mg *P.putida* Biomass by Different Potential Chemical Desorption Agents

DESORPTION AGENT	1st Sequence			2nd Sequence		
	Cadmium bound (mg)	Cadmium desorbed (mg)	% Desorption	Cadmium bound (mg)	Cadmium desorbed (mg)	% Desorption
0.1M Sulphuric acid	0.0200	0.0140	69	0.0028	0.0020	71
0.1M Nitric acid	0.0200	0.0150	75	0.0017	0.0012	71
0.1M Hydrochloric acid	0.0203	0.0200	99	0.0060	0.0050	83
0.1M Sodium hydrogen carbonate	0.0178	0.0043	24	0.0234	0.0073	21
0.1M Sodium carbonate	0.0190	0.0038	20	0.0230	0.0055	24

NB: 25ml of 1mg/l cadmium solution used in adsorption phase.
25ml of desorption agent added to recovered biomass for desorption phase.

have the same efficiency as, for example, sulphuric acid. However, citric acid might also be chelating cadmium (Gadd and Griffiths, 1978) in addition to the cadmium displacement caused by increasing hydrogen ion concentration. It should be noted though that EDTA known to be capable of cadmium chelation (Samara wickrama, 1979) was quite ineffective as a cadmium desorption agent with A.viscosus cells. This may be due to EDTA being a weaker acid than citric acid. Sodium acetate was also ineffective at displacing cadmium into solution from biomass, but had no adverse effect on subsequent biosorption.

Both sodium carbonate and sodium hydrogen carbonate were ineffective at desorption of cadmium, with sodium hydrogen carbonate being the better of the two. Both these salts significantly increased the cadmium bound by A.viscosus biomass following the first adsorption/desorption sequence. These findings may be attributed to the extremely low solubility of cadmium carbonate (Aylett, 1979), such that addition of carbonate and hydrogen carbonate results in the precipitation of cadmium from solution. Therefore most of the cadmium which may be desorbed by carbonate or hydrogen carbonate would be precipitated out of solution and not detected by polarographic analysis. Apparently enhanced biosorption following the initial loading and unloading

of cadmium from the biomass is probably due to carbonate or hydrogen carbonate being carried over with transferred biomass and thus precipitating out some of the cadmium present in solution.

These findings may be attributed to the extremely low solubility of cadmium carbonate (Aylett, 1979), such that addition of carbonate and hydrogen carbonate results in the precipitation of cadmium from solution. Therefore most of the cadmium which may be desorbed by carbonate or hydrogen carbonate would be precipitated out of solution and not detected by polarographic analysis. Apparently enhanced biosorption following the initial loading and unloading of cadmium from the biomass is probably due to carbonate or hydrogen carbonate being carried over with transferred biomass and thus precipitation out some of the cadmium present in solution.

For P.putida hydrochloric acid also proved to be the best desorption agent. The trends observed with sodium bicarbonate and sodium hydrogen carbonate were also recorded for P.putida. There was, however, a noticeable difference between the performance of mineral acids in the second sequence of desorption, where desorption of the A.viscosus biomass was far less efficient by mineral acids than that achieved with P.putida.

Owing to the problems associated with use of carbonate or hydrogen carbonate to desorb cadmium, one of the acid desorbents was selected for subsequent desorption experiments. Although citric acid seemed better overall than any of the mineral acids used to elute cadmium, the wider availability of mineral acids in industry makes them a more attractive proposition when use of a scaled-up cadmium recovery process is considered. Thus the best of the mineral acids - hydrochloric acid - was used in subsequent experiments. It is significant that although both these bacterial species were demonstrated to accumulate cadmium intracellularly, good cadmium recovery could still be achieved.

3.3.2 Desorption kinetics

The rate of cadmium elution from K.aerogenes 9128, A.viscosus, P.putida and P.cruciviae by 0.1M hydrochloric was monitored for sixty minutes. All biomass samples were exposed to 100ml 5mg/l cadmium solution prior to acid washing. From figures 33-36 it is apparent that for each type of biomass the maximum level of desorption achievable is attained within 10 minutes, following which little or no increase occurs in the level of cadmium in solution.

FIG.34 KINETICS OF CADMIUM DESORPTION FROM 30mg
PS.CRUCIVIAE

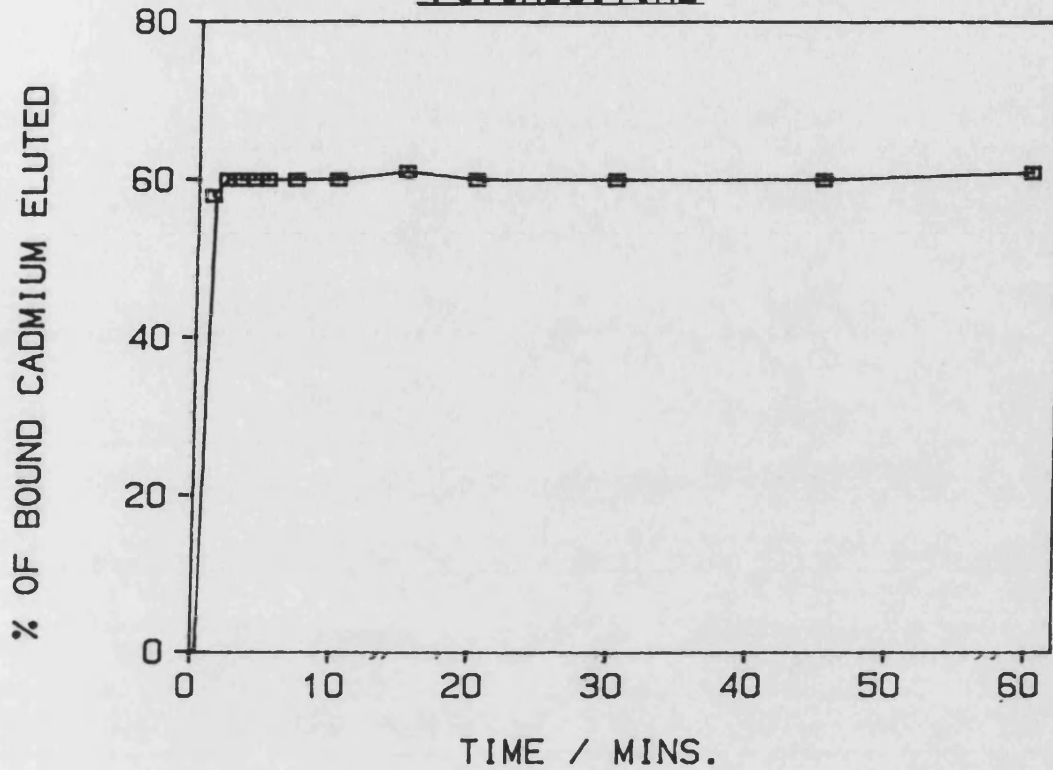


FIG.35 KINETICS OF CADMIUM DESORPTION FROM 30mg
K.AEROGENES 9128 BIOMASS

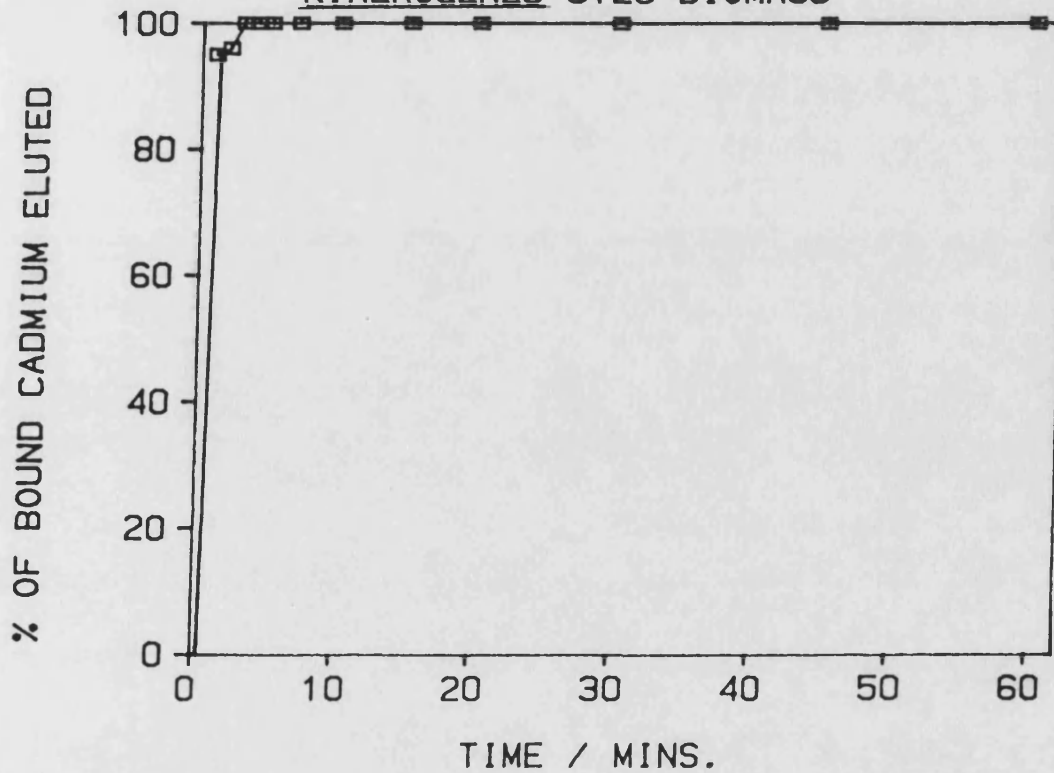


FIG.36 KINETICS OF CADMIUM DESORPTION FROM 30mg
A.VISCOSUS BIOMASS

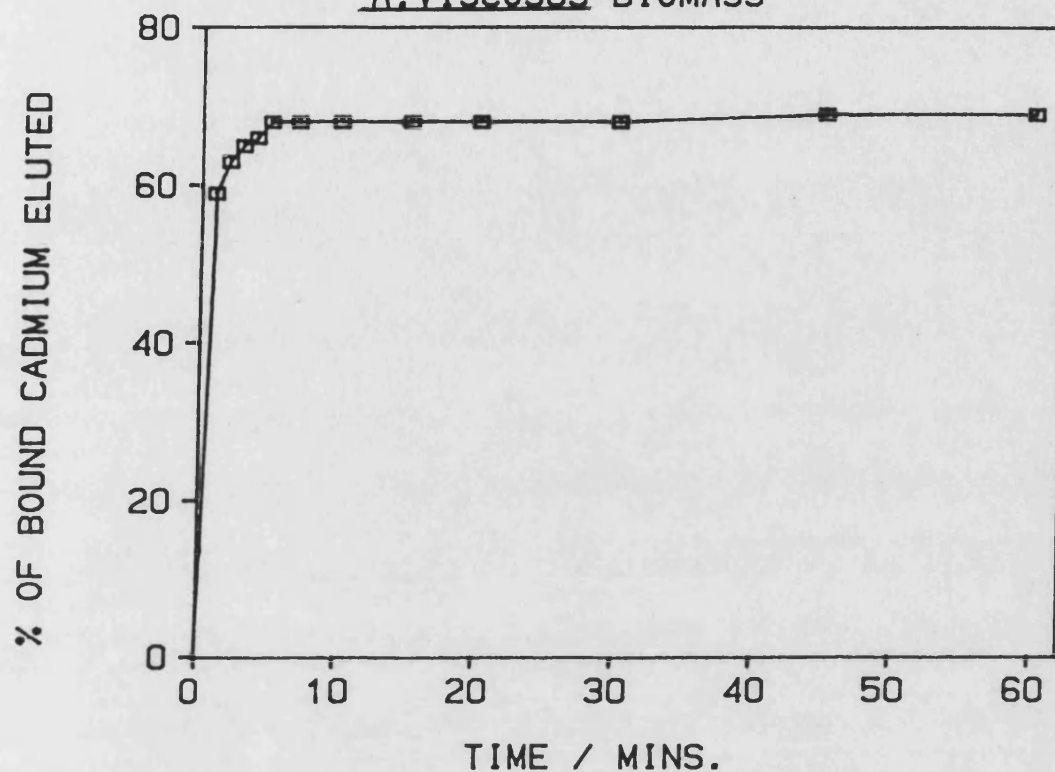
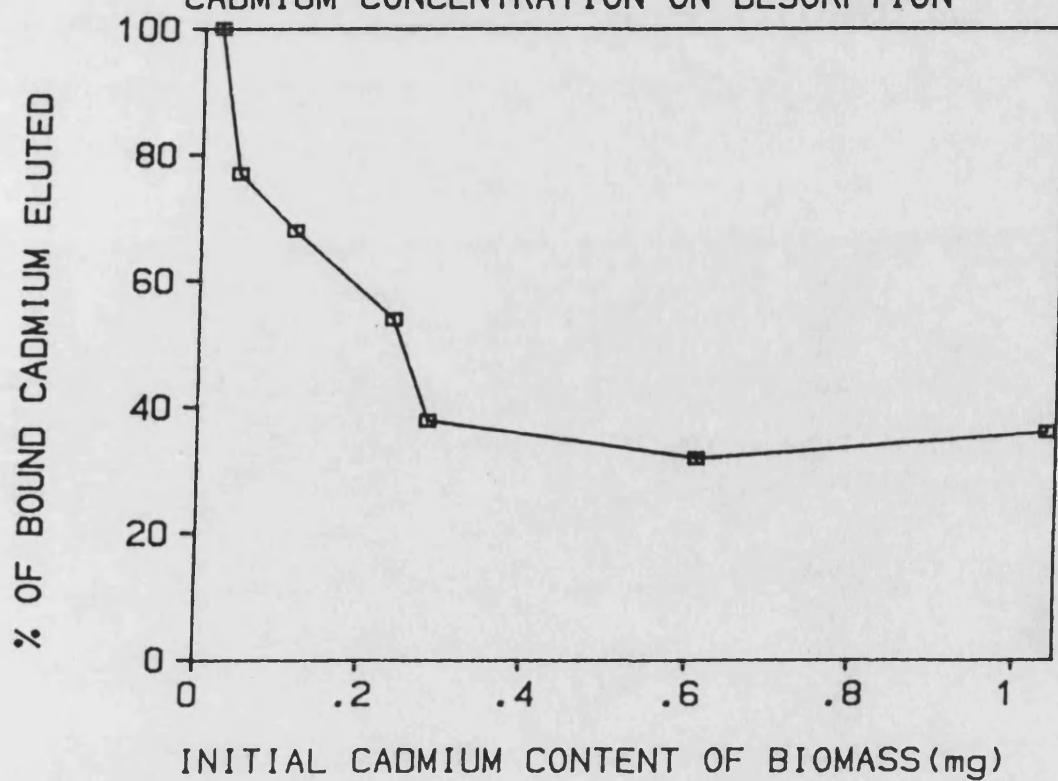


FIG.37 EFFECT OF INCREASE IN A.VISCOSUS BIOMASS
CADMIUM CONCENTRATION ON DESORPTION



3.3.3 Effect of cadmium concentration on desorption

The effect of increased cadmium loading on A.viscosus, P.putida and K.aerogenes 9128 on desorption of the cadmium by hydrochloric acid was investigated over the range 1 to 200mg/l Cadmium (Figures 37-39) .

Biomass was exposed to 25ml of cadmium solution of a particular concentration for 10 minutes, then recovered and resuspended in 25ml 0.1M hydrochloric acid for 10 minutes at which point the cadmium content of the acid wash solution was determined. An exception was K.aerogenes which was exposed to 15ml cadmium solution, then 15ml acid as a lower volume but higher speed centrifuge was required for full recovery of the polysaccharide encapsulated cells.

In each case, the proportion of cadmium removed by acid wash decreases as the amount of cadmium bound increases. It should be noted that the effect appears less marked with K.aerogenes 9128, which may be due to the lower levels of cadmium present initially.

In addition to these results, successive loading of A.viscosus cells at three different biomass levels all showed an initially sharp decline (which was more notable at higher biomass levels) as binding sites for cadmium became occupied. This was followed by a more

FIG.38 EFFECT OF INCREASE IN P.PUTIDA BIOMASS
CADMIUM CONCENTRATION ON DESORPTION

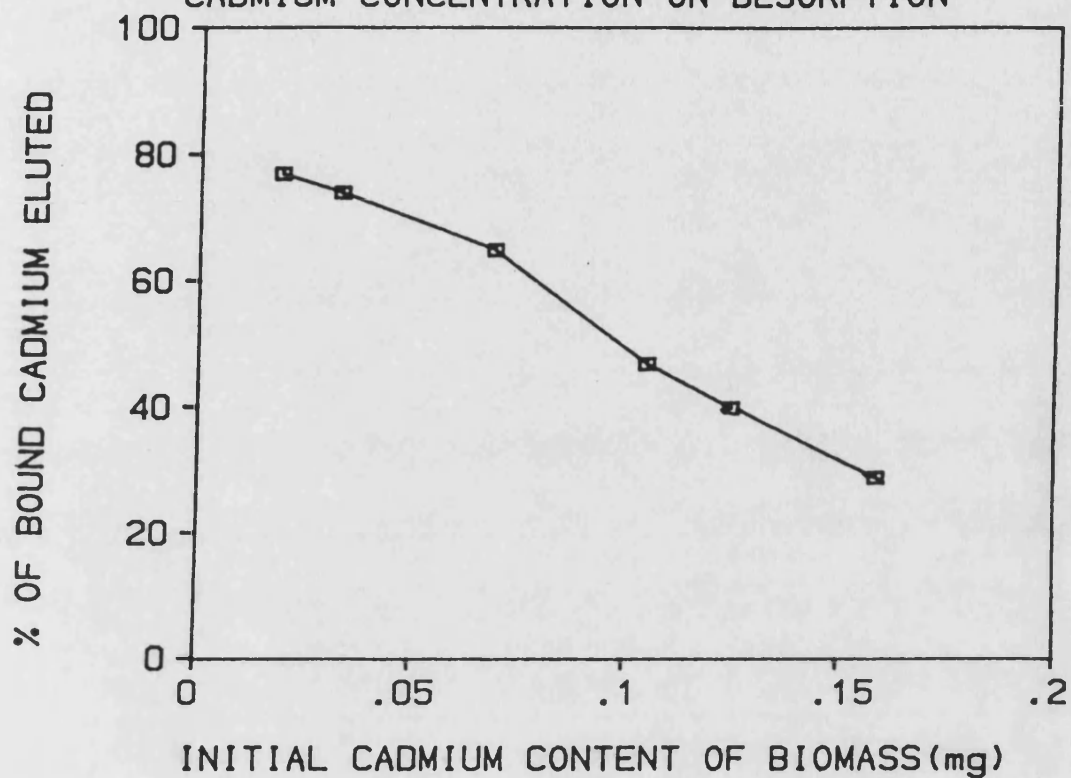
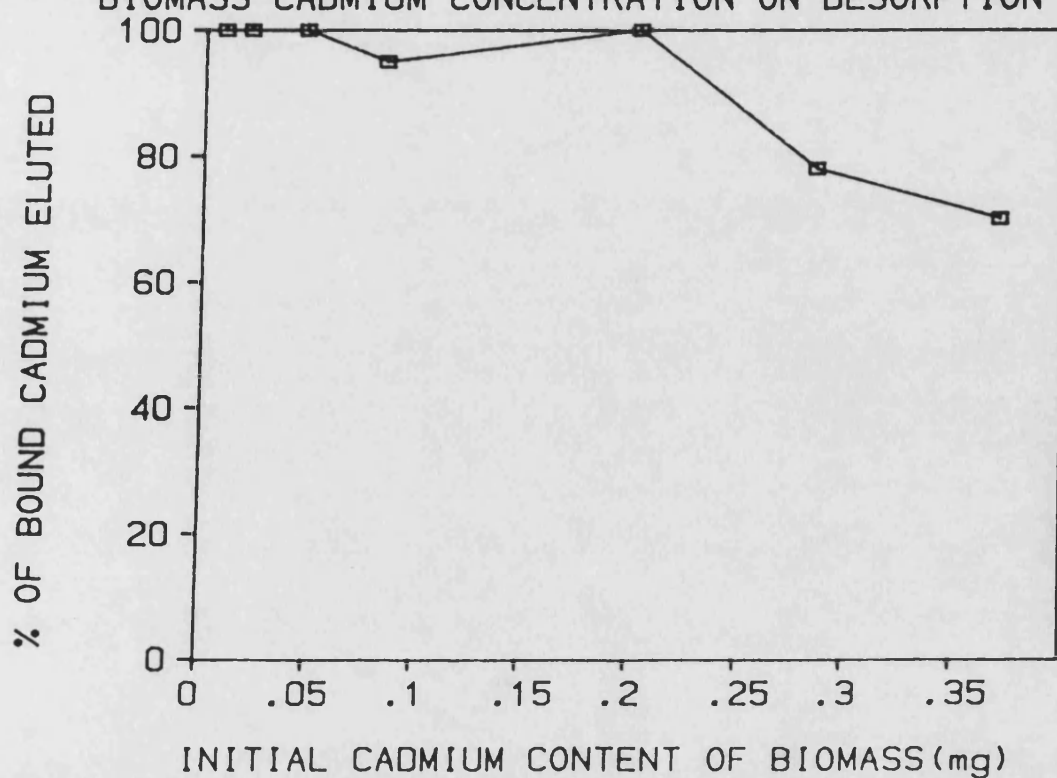


FIG.39 EFFECT OF INCREASE IN K.AEROGENES 9128
BIOMASS CADMIUM CONCENTRATION ON DESORPTION



gradual decline with successive loading of A.viscosus with cadmium (Figure 40).

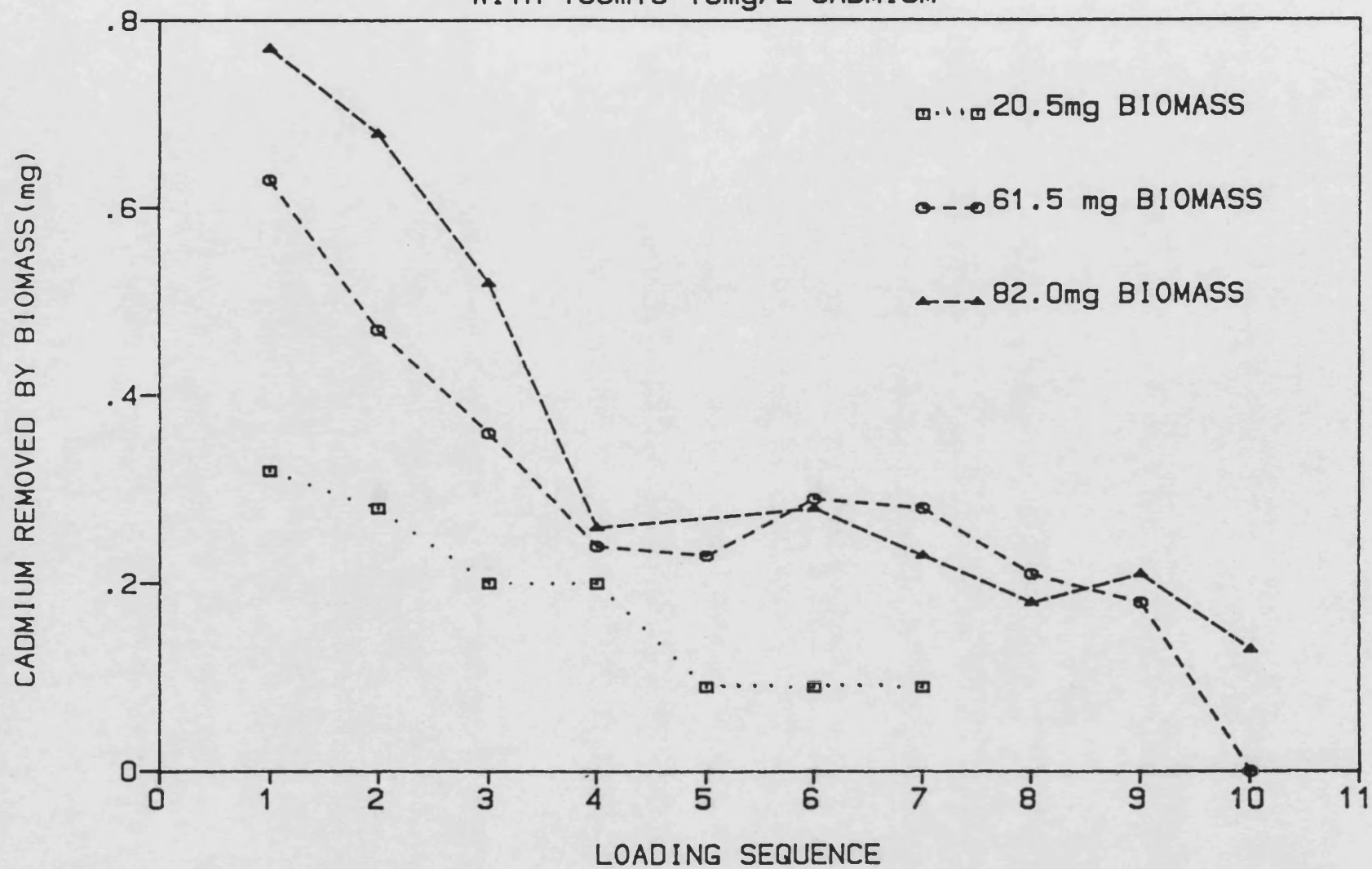
This data suggests that the mechanism of cadmium uptake of A.viscosus may alter as available binding sites become occupied. Two ways in which this may have occurred are:

(i) the initial steep decline in amount of cadmium bound to A.viscosus (Figure 40) being due to binding of cadmium to cell surface and other readily available sites. The subsequent almost constant level of uptake being achieved by the phosphorus linked cadmium detoxification system previously described (3.2.9).

(ii) the initial steep decline in amount of cadmium bound being due to depletion of the phosphorus compound (perhaps present as phosphate) postulated to bind cadmium locally. Subsequent metal uptake being achieved by active uptake.

To ensure presence of enough cadmium to be detected by x-ray spectroscopy, cells were exposed to 1000mg/l cadmium solution whereas the successive exposure experiments used 10mg/l cadmium solution. It seems quite likely that the amount of intra-cellular uptake by A.viscosus is not so extreme under

FIG.40 SUCCESSIVE LOADING OF A.VISCOSUS BIOMASS
WITH 100mls 10mg/L CADMIUM



the successive exposure to cadmium experiments. As desorption of cadmium was less effective with larger amounts of bound cadmium, it is most likely that mechanism (1) operates within A.viscosus. Surface bound metal would be more easily eluted than intracellularly bound metal. In addition, A.viscosus has not been shown to exhibit the degree of active accumulation of cadmium sufficient to account for the level of cadmium bound in Figure 40, from the 4th loading exposure sequence onward, particularly as prolonged cadmium exposure and cell transfer decreases viable cell numbers within the population.

Desorption of cadmium from K.aerogenes may thus be more effective at higher cadmium loadings than that observed for A.viscosus and P.putida because K.aerogenes does not bind cadmium intracellularly.

3.4 Fluidised bed experiments

3.4.0 Cadmium biosorption by biomass in a fluidised bed
Of the biomass types examined during the course of investigation, A.viscosus and K.aerogenes had the most favourable isotherms for cadmium removal from solution, with A.viscosus being particularly efficient between 1 and 10ng/l cadmium. However, with regard to process application on a larger scale, A.viscosus possesses the

disadvantage that exopolysaccharide produced is soluble and most would therefore be carried away in solution with any cadmium it accumulates. Whereas, that of K.aerogenes 9128 is contained in a capsule surrounding the cell wall. Even though the A.viscosus exopolysaccharide produced and recovered under benchscale experimental conditions was only approximately 3% of the total biomass weight, it would still represent an unacceptable loss of bound cadmium from the bioreactor if the process were translated onto a large scale. Furthermore, A.viscosus has been demonstrated to bind cadmium intracellularly and, more importantly, to be less efficient for cadmium recovery at higher cadmium loadings.

However, other factors need to be considered with respect to application of the biosorption process. The object of the fluidised bed trials was to identify and to evaluate the critical factors involved in running a cadmium recovery process using live biomass in a fluidised bed. Arthrobacter species are generally quite robust (Keddie and Jones, 1983), so the majority of experiments were carried out with A.viscosus. Later experiments used K.aerogenes 9128 as biosorbent.

During the course of the trials three different fluidised beds were constructed. Each had the same

basic design with one version having a dual column system. These fluidised beds will be referred to as Beds I, II and III, in chronological order of construction and operation. Beds I and III were constructed predominantly of ABS plastic fittings claimed to be less adsorbent than PVC); bed II was composed of QVI glass fittings. Details of size and construction are given in Chapter 2 (Materials and Methods).

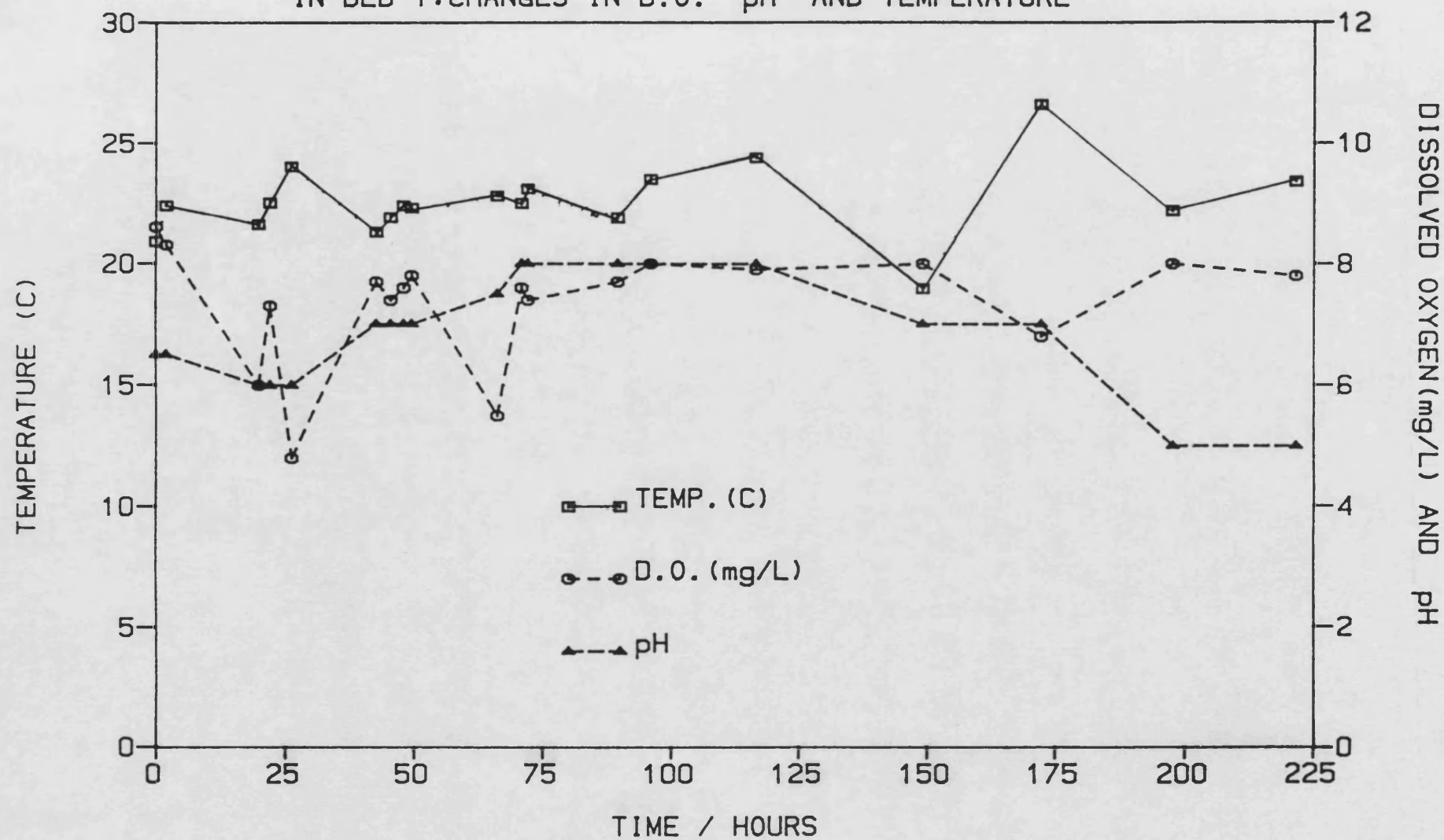
3.4.1 Growth of A.viscosus in a fluidised bed

Growth of A.viscosus on sand support (Materials and Methods) was monitored and efficiency of cadmium uptake of the sand associated biomass evaluated. The pH, dissolved oxygen concentration and temperature within the biosorption column were noted over a period of ten days. At seven points during the course of the run samples of sand plus associated biomass were withdrawn from the uppermost sample port on the column and the amount of biomass attached to the sand was determined ; this was carried out 22, 48, 72, 96, 116.5, 149 and 197.5 hours after inoculation. Prior to dry weight determination sand-associated biomass was exposed to cadmium solutions of 12.5 and 25.0mg/l. Microscope slides were prepared of sand-colonising and medium-containing cells every 48 hours, along with a sample of medium to determine the viable count of cells.

Figure 41 shows the fluctuations in dissolved oxygen content (DO), pH and temperature of the medium passing through the bed column. Temperature varied diurnally reflecting fluctuations in laboratory ambient temperature. No temperature control existed within the system, but the fluctuations in temperature were not excessive (with a maximum recorded of 27.6°C; minimum 19.0°C).

As would be expected, rise in temperature caused a corresponding drop in the dissolved oxygen content of the medium. However, at two points during the trial a sharp drop in DO occurred beyond what might be explained by increase in temperature. The first of these abrupt drops in DO precedes a peak in viable cell count (Figure 41) at 48 hours. Microscope examination of the culture at 22 and 40 hours and observation of colony morphology on spread plates, indicated that no other contaminants had become established within it. It must be emphasised however that such general techniques unsupported by a number of specific nutritional tests are incapable of identifying any contaminating microorganisms precisely, and only provide a general overview of the cell population. Nevertheless, up to 48 hours no other organism apart from A.viscosus were isolated. Thus the rise and peak in viable cell numbers can be associated with

FIG41 CADMIUM UPTAKE BY SAND ASSOCIATED A.VISCOSUS
IN BED 1:CHANGES IN D.O. pH AND TEMPERATURE



growth of A.viscosus, and the rapid drop in DO may also be attributed to rapid growth of such an aerobic bacterium.

A sample taken for microscope analysis and spread plating at 48 hours, following the drop in DO revealed the presence of a contaminant that was a Gram positive coccus and formed pink colonies on nutrient agar. The contaminant became established within the system over the next few days at the expense of A.viscosus, as revealed by the colonies formed on viable count spread plates. However, note that the viable count of Figure 42 is a record of the total cell population, not a breakdown of the colony count from the spread plates. In other words, no differentiation was made between colony types counted on spread plates.

At the point which the dissolved oxygen content of the medium reached a minimum the medium pH also began to alter. This rise in pH was probably connected to growth of the contaminant bacterium at the expense of A.viscosus. The establishment of the contaminant did not directly cause a drop in cell population on the sand grains which comprised the support material (Figure 42), but did cause a drop in the specific binding capacity of the sand associated biomass (Figure 43) at both cadmium concentrations tested from 48 to 116 hours. This may be

FIG.42 Cd UPTAKE BY SAND ASSOCIATED *A.VISCOSUS* IN
BED 1:VIABLE COUNT IN MEDIUM AND BIOMASS ON SAND

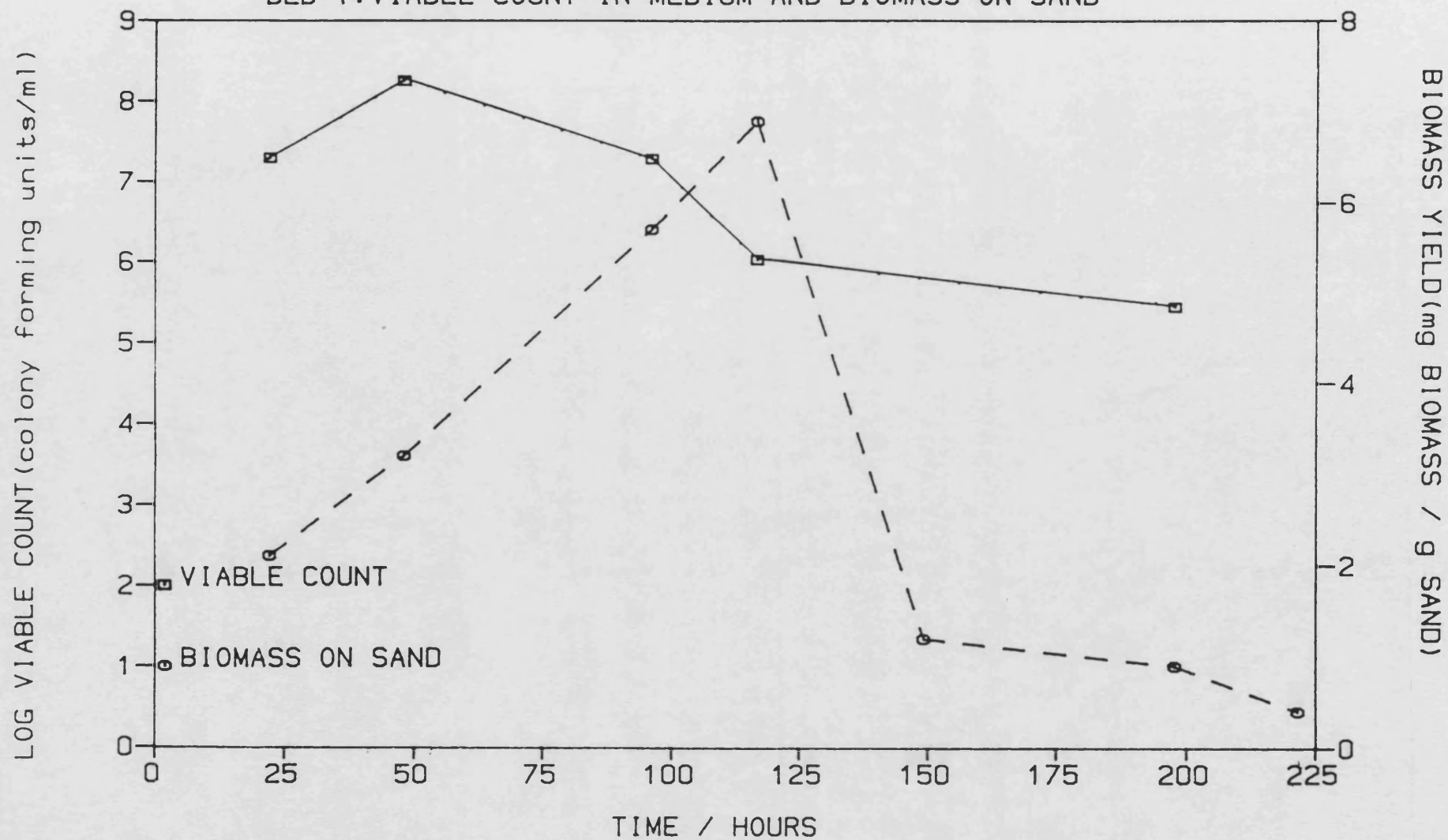
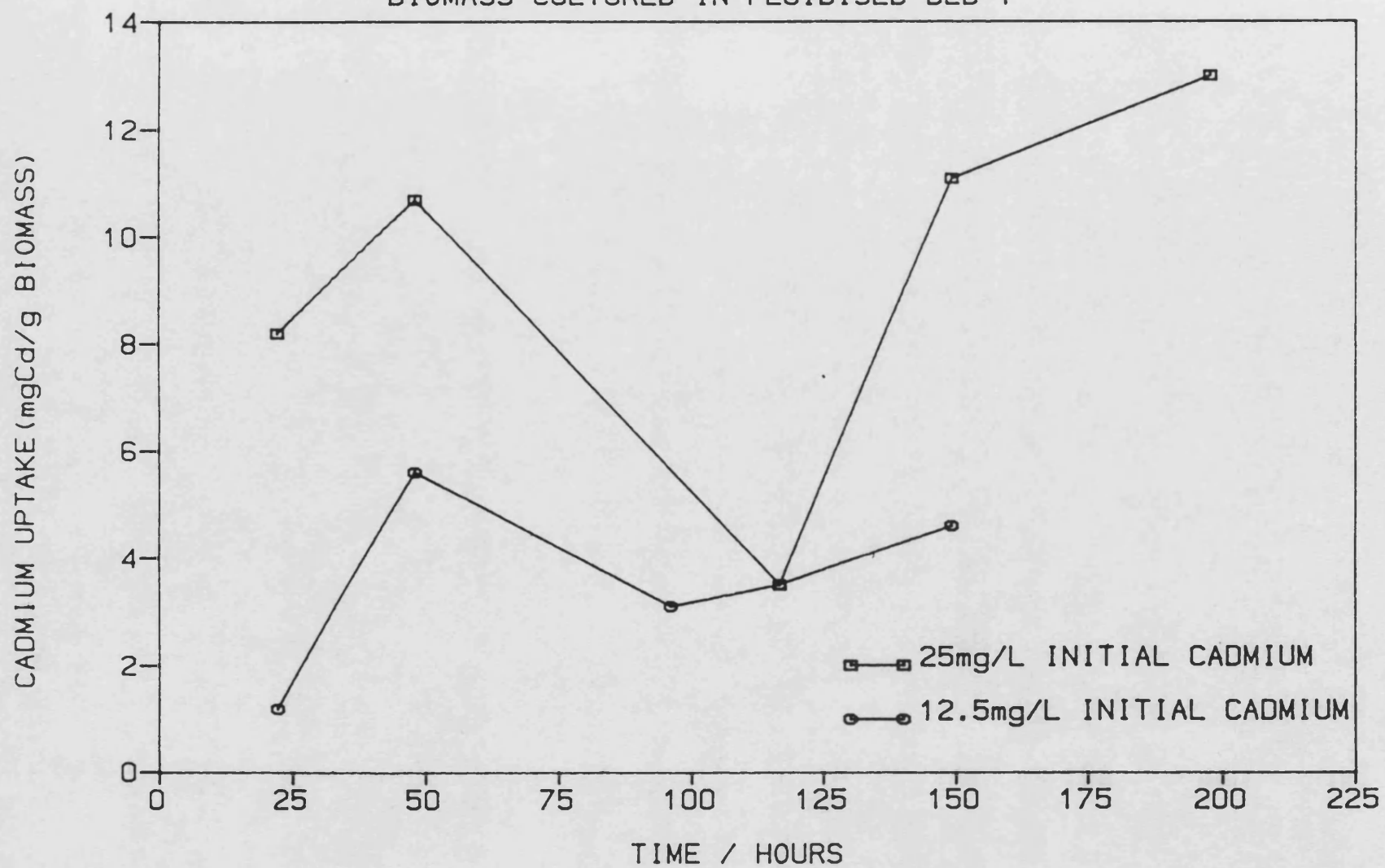


FIG43 CADMIUM UPTAKE BY SAND ASSOCIATED *A.VISCOSUS*
BIOMASS CULTURED IN FLUIDISED BED 1



attributed to some attachment of the contaminant to the support, which was not as capable of binding cadmium as A.viscosus.

Another drop in dissolved oxygen content occurred from 48 to 66 hours. This may be attributed to the growth of the contaminant isolated at 48 hours. At 116 hours into the trial, pH ceased to rise and began a decline which coincided with a sharp dropping off in the amount of biomass attached to the sand grains. Further nutrients were added to the system at 140 hours which temporarily halted the slide in pH for 30 hours until it finally declined sharply to pH 5.0. Addition of new nutrients also appeared to stem the abrupt drop in cell population on the sand grains, but only to the degree that the rate of decline became more gradual. The total viable cell population appeared to decline at a steady rate from 48 hours onward, but visual observation of the spread plates showed that from 48-96 hours the proportion of live A.viscosus cells isolated declined whilst that of the contaminant increased. From 149 hours, the contaminant population also declined and from, the pH data, possibly began to do so at about 116 hours.

At 116 hours and onward the cadmium binding by sand associated biomass for 12.5mg/l cadmium particularly

(Figure 43) appears to have increased dramatically. This was not in fact the case. As Figure 42 shows, the level of biomass associated with sand dropped off rapidly between 116 and 149 hours and this was responsible for the apparent increase in cadmium binding. As has been demonstrated previously here (Section 3.2.3), the amount of cadmium bound by biomass decreased with increased biomass concentration when the cadmium biosorption is depicted as mg cadmium bound per gramme of biomass (thus when a low biomass level is used the biosorption capacity for A.viscosus appears high). It is proposed that the course of events followed during the course of the trial were as follows.

During a period of rapid growth from point of inoculation up to 40 hours A.viscosus caused a substantial drop in the dissolved oxygen content within the system and a rise in pH. As a result, its own rate of growth declined and allowed a contaminant to become established. Between 20 and 48 hours the biomass associated with sand support was predominantly A.viscosus, and biomass growth on the sand and cadmium uptake increased in tandem. Beyond 48 hours another drop in DO occurred which coincided with growth of the contaminant

However , nutrient levels had already been

depleted by growth of A.viscosus, and A.viscosus population itself had begun to decline as the contaminant did not have adequate nutrients to achieve the population level reached by A.viscosus previously. At approximately 116 hours the growth pattern as regards the sand associated biomass again changed. As the amount of biomass attached to support had increased prior to this point, it may be assumed that the contaminant colonised the already A.viscosus-layered sand. Thus A.viscosus would be masked from cadmium solution when sand associated biomass was exposed to it and this would contribute to the drop in cadmium biosorption shown in Figure 43 (along with the increase in attached biomass).

At 116 hours the A.viscosus cells on sand were so starved of nutrients that they died and by this point the colonising contaminant, in the lowest nutrient environment, also declined. Thus the sand associated biomass levels decreased and the rate of decline was only slowed by the addition of further nutrients.

3.4.2 Uptake of cadmium by A.viscosus biomass from cadmium solution circulated within Bed I

A series of experiments was carried out in which the uptake of cadmium within bed I by the bed and sand support was assessed. In three control experiments the amount of cadmium bound during passage of a 25mg/l solution through the fluidised bed plus sand support (1kg) over 24 hours was ascertained. In a lateral consecutive trial the biosorption of cadmium within the fluidised bed by sand and bed components was assessed at 50mg/l and any biosorption caused by addition of silicon antifoam reagent and finally, standard medium constituents (Figure 44). At 25mg/l initial cadmium the bed and support only bound 2mg/l of cadmium over the first four hours of exposure. However, after 24 hours 13mg/l of cadmium had been bound. At 50mg/l cadmium binding by bed and support was again negligible within the first hour of exposure. After 6 hours 3mg/l had been bound at which point 50ml of silicon antifoam reagent were added. This reduced cadmium levels by only 3mg/l. At 7 hours dried nutrients were added to the bed reservoir (all constituent biochemicals of the standard growth medium used). By the next cadmium determination at 8 hours into the trial, cadmium concentration had dropped to 13mg/l; a drop of 31mg/l. Thus the fluidised bed in combination with sand support did not bind more than 40% of available at both concentrations examined

FIG.44A CADMIUM REMOVAL FROM 50mg/L SOLUTION IN BED 1: ADDITION OF GROWTH MEDIUM AND ANTIFOAM AGENT

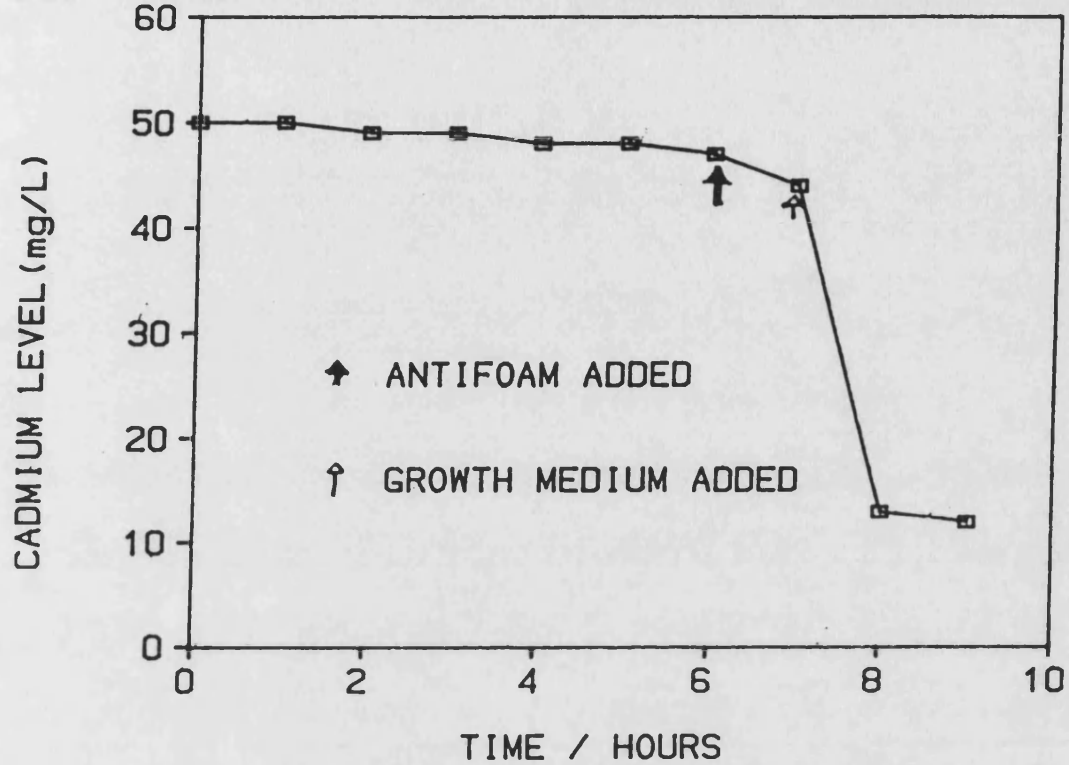
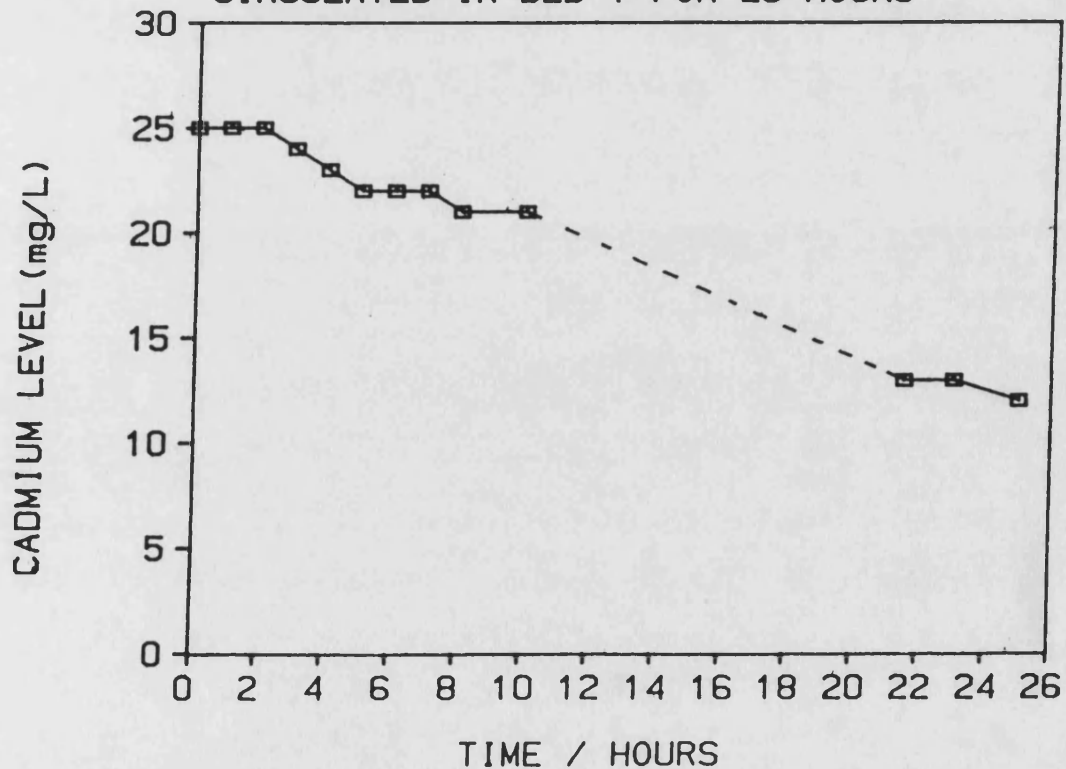


FIG.44B CADMIUM REMOVAL FROM 25mg/L SOLUTION CIRCULATED IN BED 1 FOR 25 HOURS



within 1 hour. Medium constituents bound a large proportion of available cadmium.

Following these trials a 24 hour culture of A.viscosus was prepared in Bed I and at the end of the growth period growth medium was drained out of the system and replaced by distilled water, which was circulated around the system for 1 hour to wash out any further traces of medium and loosely attached cells. During this process the flow rate was dropped to a low level (2l/min). The wash water was then drained from the bed and 20L of 50mg/l cadmium solution prepared by adding 20L of distilled water and the appropriate amount of cadmium to the bed reservoir. The cadmium solution was then circulated and cadmium removal monitored over 60 minutes (a period when bed and support cadmium binding was negligible). From Figure 45 it is apparent that 60% of available cadmium was bound within 3 minutes exposure. Following this the level of cadmium present remained stable at 21mg/l over the next 57 minutes of monitoring. After the trial, sand was abstracted from the fluidised bed and some was subjected to acid desorption treatment. Other sand samples were taken to determine the amount of biomass associated with sand. The average was found to be 1.10mg biomass per gramme of sand, a total of 1839.8mg biomass retained by sand within the system which bound overall 580mg of cadmium; an overall binding

FIG.45 CADMIUM UPTAKE FROM 20L OF A 50mg/L SOLUTION IN BED 1 BY SAND ASSOCIATED A.VISCOSUS

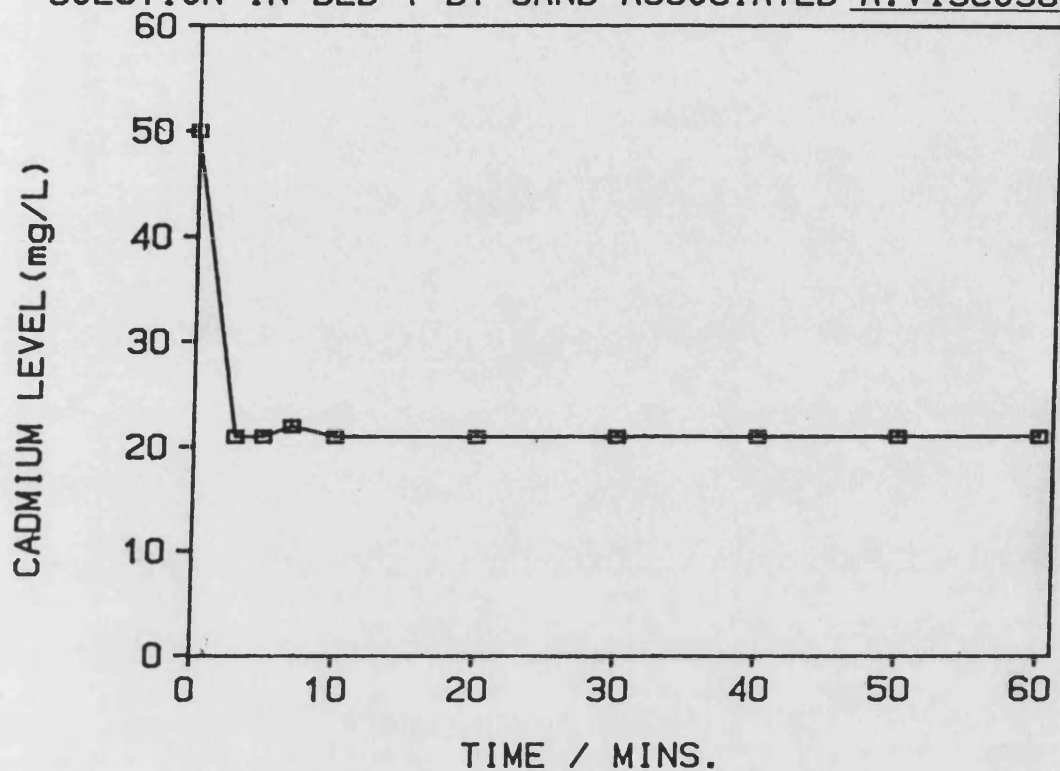
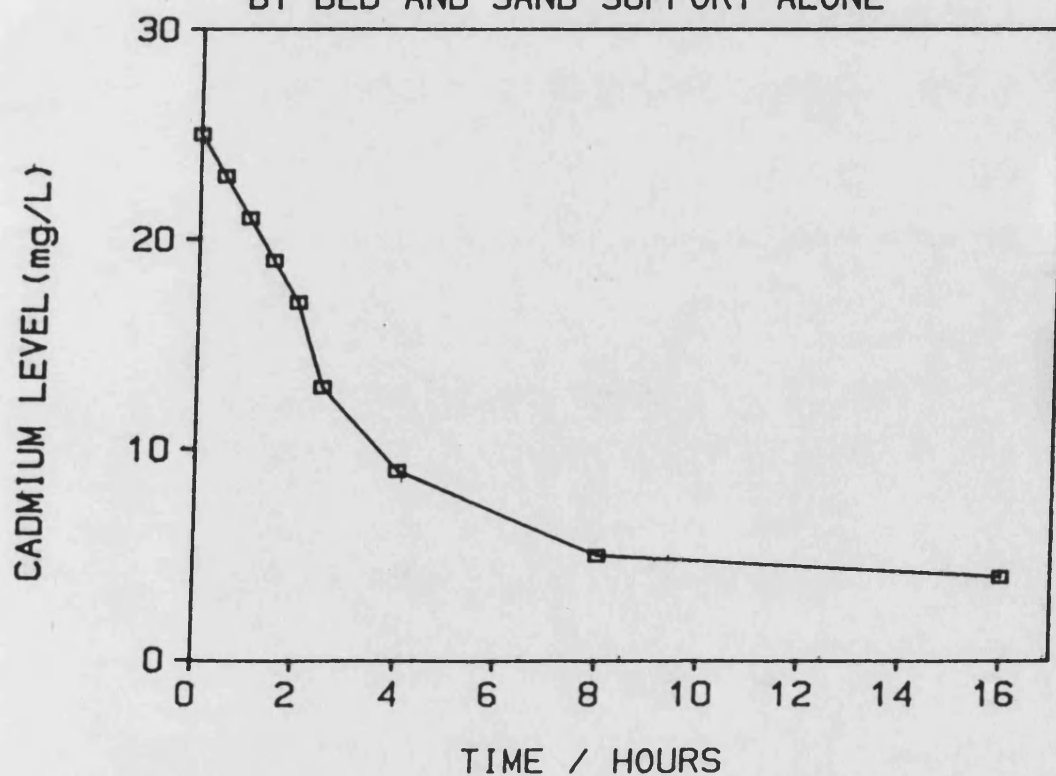


FIG.46 CADMIUM UPTAKE IN FLUIDISED BED2: UPTAKE BY BED AND SAND SUPPORT ALONE



capacity of the A.viscosus biosorbent under these conditions of 315mg cadmium/g biomass. However, allowing for 4% of uptake to be accounted for by bed and support material together, uptake was still far above what might be expected corrected for support and bed uptake was 302.6mg cadmium/g biosorbent. Further investigation revealed the probable cause of this discrepancy. When samples of fluid from the reservoir of the bed were examined they were found to contain biomass; a dry matter determination established that 2045.6mg of free (i.e. unattached) biomass was present in the system. This gave an adjusted uptake of 143mg cadmium per gramme biomass; still high, but probably attributable to formation of a slime layer on the reservoir walls during the original growth sequence which had not been removed during the wash procedure. This particular trial uncovered two very important points. The first was that under the course of a cadmium uptake trial with a fluidised bed, if the cadmium uptake by sand attached biomass is determined within the whole system the attrition of biomass from support, and the presence of any unwanted colonies of cells at points in the system must be taken into account. Thus the previous method of sampling employed in the 10 day experimental trial where biomass on support was exposed to cadmium outside of the bed gave a more accurate measure of the binding capacity of the sand attached biomass.

The second point to arise from this trial is that 53% of the biomass in the system that was determined by dry matter analysis was actually not in contact with the support. This raises serious doubts about the value of using sand-attached biomass in a fluidised bed for cadmium recovery.

3.4.3 Assessment of A.viscosus biomass attachment within fluidised bed II on different support materials

Following the discovery of substantial amounts of A.viscosus biomass being stripped from sand grain support during a cadmium biosorption trial, a series of experiments was conducted on cadmium biosorption and attrition of biomass from support material for several different support materials.

The fluidised bed employed during this series of trials was constructed of QVF glass fittings. The biosorption column containing the support was a QVF glass column of 1 1/2" bore internal diameter. Aeration of both reservoir and column were carried out during liquid culture period. The working capacity of the system was 15l. Uptake by the glass system was determined in a control trial and found to be greater than that of the ABS plastic bed I (Figure 46). No support material was

included in this trial. Over the initial 60 minutes of cadmium circulation, cadmium uptake within the fluidised bed was 6% higher than that with the ABS plastic bed I. Furthermore, over sixteen hours of the trial the cadmium concentration declined far more rapidly in bed II than the bed constructed from ABS bed I (compare Figure 44(b) with Figure 46). This may be attributed to biosorption by the glass surfaces. This phenomenon has been noted by, amongst others, Strandberg et al (1981) and was avoided in bench scale experiments by coating glassware used with a silicone compound (see Materials and Methods). However, metal ion adsorption to glass occurs over a far longer period than cadmium biosorption than microorganisms, and even in bed II, composed predominantly of glass fittings, cadmium biosorption over the first 60 minutes of exposure only amounted to 6% of available cadmium. In comparison cadmium biosorption by bacteria observed during this course of study occurred rapidly; and equilibrium was usually reached after 10 minutes exposure to metal solution. Support materials used included sand, gravel, perspex tubing, viton tubing, ceramic beads and glass beads (Materials and Methods).

In Table 20 the results of these trials are presented with the amount of biomass removed during treatment steps and that remaining on support presented in terms

TABLE 20: *A. viscosus* Biomass Retention on Support Material Through Biosorption and Desorption

		SUPPORT TYPE					
		Sand	Gravel	Viton Tubing	Perspex Tubing	Glass Beads	Ceramic Beads
% of Total Biomass in or on	Rinse Fluid	35	47	27	56	59	18
	Biosorption Fluid	12	15	17	15	13	11
	Desorption Fluid	16	26	23	22	23	32
	Support Material	37	12	33	7	5	39
	Total	100	100	100	100	100	100

of the percentage of the total amount of biomass present. In all cases over half the biomass originally present in the system was lost, and in most cases the bulk of material lost from the system was lost during the initial rinse. Considering the fact that acid washing could be considered more rigorous, the biomass initially lost would probably be that least well attached. In all trials the desorption stage with acid was responsible for more biomass loss than the bio-sorption stage. Presumably, with the least well attached biomass washed away during the rinse stage, more biomass was lost during acid treatment due to damage inflicted on cells by acid.

Of all the support materials, perspex tubing and glass beads fared worst, probably because they had quite smooth surfaces. During the acid wash with gravel the circulating solution turned black as some unknown chemical compound was released from the gravel. This might explain the poor adhesion experienced with gravel. Porous ceramic beads presented the best support for biomass, probably because the porous structure might provide some shelter. However, sand and viton tubing also functioned reasonably well.

Overall, none of the support materials was satisfactory as during the course of treatment all support types lost

over 50% of the biomass in the system. The figures might be distorted by washout of clumps of biomass from colonised structures within the system, but overall it would appear difficult to retain live A.viscosus on a support material. This tends to invalidate a biosorption system based on viable material attached to biomass.

3.4.4 Cadmium biosorption within a fluidised bed by K.aerogenes 9128

Three experimental trials were carried out with K.aerogenes 9128 in fluidised bed III. Bed III, as bed I, was composed of ABS fittings with the columns containing the support made of perspex (Materials and Methods). The cadmium uptake of bed III followed the same pattern as that of bed I; within the first 60 minutes of biosorption only 2mg/l cadmium from a 50mg/l solution were bound in the bed.

1kg of ceramic beads was used as K.aerogenes support in each trial. The growth period allowed was 48 hours. Figure 47 presents the average biosorption observed over the three trials.

FIG.47 CADMIUM REMOVAL FROM 50mg/L SOLUTION IN BED
3 BY SUPPORT-ASSOCIATED K.AEROGENES 9128

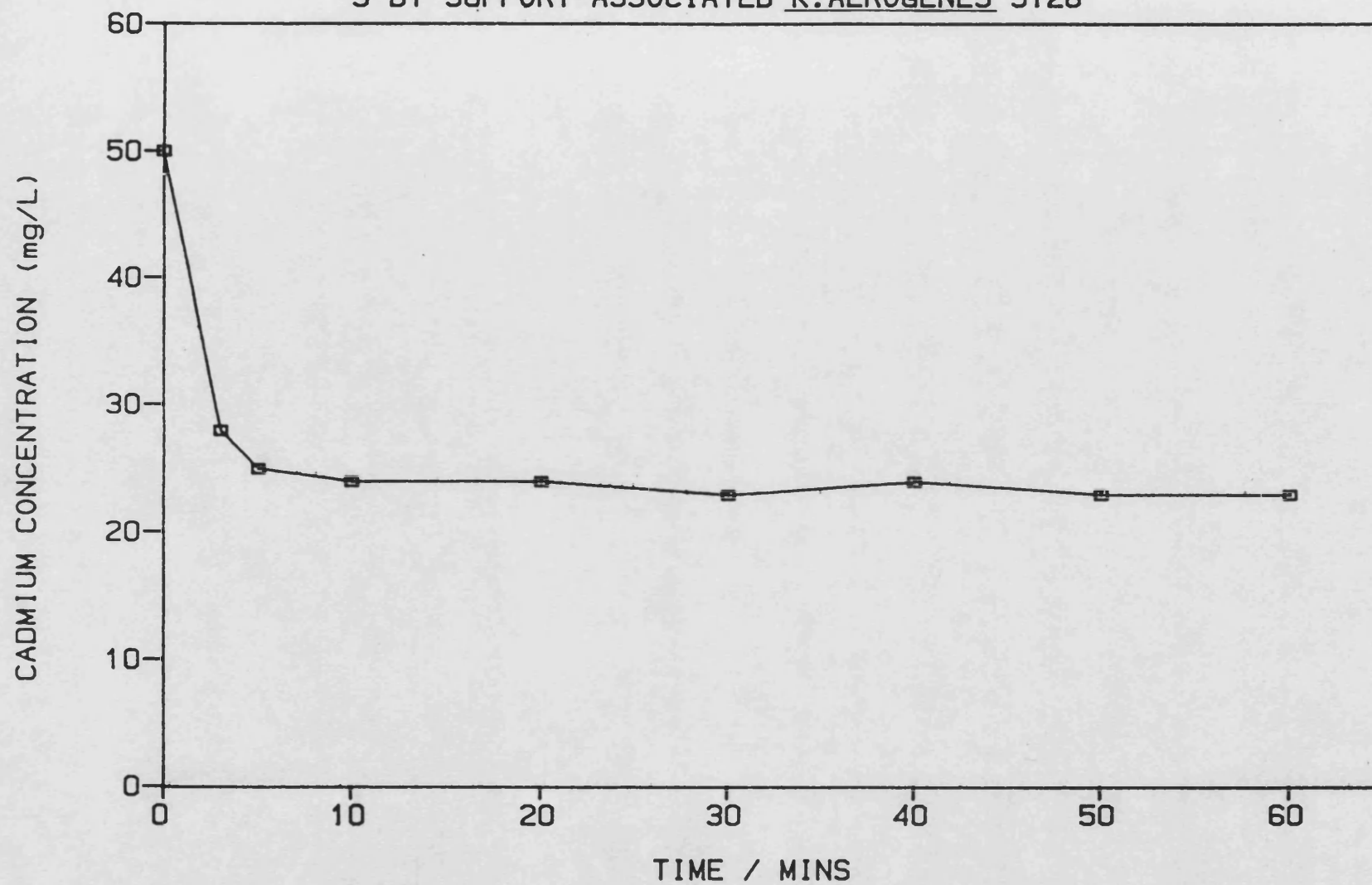


Table 21: K.aerogenes 9128 biomass retained on support

Biomass lost			
from support(g)	13.3251	14.5210	11.7765
*Biomass			
retained (g)	8.2006	7.9543	6.9534
<hr/>			
% Retained	62	55	59

*NB: No desorption with acid was carried out. Biomass retained represents that still associated after a rinse period and circulation of cadmium solution.

Biomass retention on ceramic support was considerably better than that for A.viscosus, but a considerable proportion of biomass was still lost. Cadmium biosorption at this concentration (50mg/l) was not, however, as good as that for A.viscosus, but again the loss of biomass from the support material during solution circulation places the usefulness of the technique in some doubt.

4. Conclusions.

4. CONCLUSIONS

In some of the published literature on biosorption various biomass types have been presented as suitable biosorbents for the particular metal , or metals , being investigated on the evidence of metal uptake from single - ion solutions alone . This is adequate for determination of the optimum capacity of a biosorbent but ultimately of little use by itself when attempting to establish a biosorbent as a better material for heavy metal recovery from waste-water than ion exchange resins or , representing a better metal recovery process than conventional processes (for example , precipitation , electrolysis , e.t.c.) .

From this course of study , it is clear that the metal uptake capacity of a particular bacterial biosorbent is is dependent on many factors . Thus , with regard to use of a biosorbent all factors which might affect its binding capacity (such as pH , temperature , ionic competition , sites of binding and ease and efficiency of metal recovery) need to be taken into account before the type of biomass employed can be properly assessed . Furthermore , the potential of a biosorbent is still only partially assessed when these criteria have been satisfied . Biosorbent needs to be employed in a contacting device to give any reasonable assessment of its ability to compete with existing waste-water metal removal processes and finally give an

indication of the operating costs of the system .
During the time allowed for this study only preliminary investigations into operation of a proposed cadmium recovery system in a fluidised bed with live biomass were carried out but these were enough to identify the main operating problem with the system (attrition of biomass from support) .

As mentioned previously (section 3.1.2) ,when assessing the optimum metal uptake capacity of a biosorbent it is important to clarify what amount of metal (and what amount of biosorbent) is used if uptake capacities are to be compared with data in the literature . Concentration terms , where used , should be given in conjunction with the volume used in any presentation of the data .

Under the experimental conditions employed cadmium uptake by A.viscosus and K.aerogenes strains 9218 and 9527 compared favourably with cadmium uptake capacities available in the literature (1.5.3) .

Cadmium uptake by the bacterial species used fitted either the Langmuir or Freundlich adsorption isotherms well enough between 0.05 and 20.0 mg initial cadmium for the uptake to be described as adsorption or, in this case, biosorption. Only A.viscosus and A.globiformis had cadmium uptake over the range 0.05-100mg (0.5-1000 mg/l) assessed.

Over this greater initial concentration range neither Langmuir nor Freundlich isotherms fit the data with the required degree of significance. Nevertheless, over the lesser concentration range the accumulation of cadmium could be described as adsorption. Such a relationship is significant with regard to development of a waste-water treatment process utilising biosorbent.

To realise fully the potential of a promising metal accumulating microbial strain, the mechanisms that control the process of metal uptake need to be elucidated and the factors affecting uptake identified. Throughout this study biomass was still viable when exposed to metal. This increases the importance of adequately characterising the biosorbent as live cells might have metabolically regulated systems that affect their metal binding capacity. The paradox associated with live microorganisms for cadmium removal is that many of those most tolerant to toxic heavy metal may be so through systems developed to decrease uptake of metal. Propagation of live biomass outside the waste-water stream is the standard strategy for meeting this problem.

If metabolic and other active uptake systems are disregarded, heavy metal ion accumulation by cells may be described as a physio-chemical process which could be

either adsorption or complexation. Microbial cells are capable of both. At present no set definition exists for the process termed biosorption. Shumate and Strandberg (1985) define biosorption as a "non directed, physical-chemical interaction between dissolved metal species and charged cellular components", similar to ion-exchange. Tsezos (1986) describes biosorption as occurring via sequestration of metal by entrapment, cation exchange, complexation, active transport or adsorption. The approach taken in this study is the use of the term biosorption where uptake of metal could be proved to fit an accepted pattern of adsorption. This does not mean to say that metal uptake that follows a Langmuir adsorption isotherm may be due to adsorption in the strictest sense; Sterritt and Lester (1986) point out that binding of heavy metals to activated sludge flocs follows a Langmuir isotherm, but the mechanism is more strictly a complexation.

In terms of process application, the characterisation of biosorbent needs to achieve enough for some predictive capability for its performance to be attained. A biosorbent needs to achieve rapid metal binding, bind a comparatively large amount of metal and allow easy recovery of metal; all without the need to subject the biosorbent to too much processing which would reduce the cost-effectiveness of using microbial cells.

All the bacterial species employed showed rapid cadmium accumulation. At low concentrations (i.e. 0.5-10mg/l), A.viscosus appeared the most efficient biosorbent, whilst at higher concentrations (10-100mg/l initial cadmium) K.aerogenes strains proved most effective. Although K.aerogenes strains 9128 and 9527 which produced extracellular polysaccharide as a capsule around the cell appeared to bind more cadmium than strain 9528, the difference was not major. In addition, investigation into the contribution that extracellularly excreted polysaccharide of A.viscosus made to the bacterium's cadmium biosorption showed that under the conditions used, the polysaccharide's contribution was slight. This was confirmed by electron microscopy which revealed that cadmium biosorption by A.viscosus resulted predominantly in localised intracellular accumulation which was associated with phosphorus. Some binding of cadmium to the cell wall still probably occurred though. Strandberg et al (1981) found that formaldehyde treatment enhanced uranium biosorption and postulated that this might occur through lowering of the amount of positive charge on the cell wall. If this is indeed the case, then some surface binding of cadmium did occur with A.viscosus and with the other bacterial species used which showed enhanced cadmium uptake following formaldehyde treatment. Some caution also needs to be

exercised in interpreting the electron microscope data as a cadmium concentration of 1000mg/l was employed to ensure enough cadmium uptake to be distinguished by x-ray spectroscopy. At this level of initial cadmium other mechanisms of cadmium uptake, apart from those occurring at lower concentrations, might appear - the Langmuir isotherm did not provide a significant fit for A.viscosus at this level of initial cadmium.

Cadmium biosorption by A.viscosus was not greatly enhanced by exopolysaccharide production under these conditions. However, it should be noted that the polysaccharide produced by A.viscosus had, weight for weight, a significantly greater cadmium binding capacity than the cells themselves.

Polysaccharide produced by K.aerogenes 9528 was not bound around the cell as a capsule but excreted into the medium (as occurred with A.viscosus). The two capsule forming polysaccharide producers - K.aerogenes 9128 and 9527 - both exhibited adsorption isotherms which showed them to be better biosorbents than strain 9528. However, it should not necessarily be concluded that presence of a capsule improves cadmium biosorption by these strains (9527 and 9128). A complicating factor is that electron microscopy and x-ray spectroscopy did not provide direct evidence that this was the case, but did

seem to indicate that all these strains had a high sulphur content, perhaps linked to one of the detoxification systems observed by Aiking et al, (1982), (although there are reasons for this not being the case: Section 3.2.9). Capsular production by polysaccharide producing K.aerogenes has been found to increase heavy metal tolerance of the cells (Bitton and Freihofer, 1978; Bauda and Block, 1985) which is presumed to be due to the polysaccharide of the capsule binding cadmium and preventing it being taken up into the cell. At present, all that may be safely concluded is that the polysaccharide extracellular capsule of K.aerogenes strains 9128 and 9527 mediates in cadmium uptake.

Microbially produced polysaccharide itself has great potential as a metal biosorbent. A.viscosus exopolysaccharide contributed little to biomass cadmium uptake in these investigations because the cells produced little polysaccharide, or had little polysaccharide transferred with them, under these experimental conditions. The polysaccharide itself showed greater biosorptive capacity for cadmium than any of the cells used. Microbially excreted polysaccharide is more homogenous than the producing cells which would allow it to be better characterised. In addition, if microbially produced polymer were used as biosorbent, the problems associated with using live biomass would be

circumvented. Cell-excreted polymer is produced at costs not significantly above those for cell propagation. Apart from A.viscosus, other bacteria are presently used in industry to produce complex polysaccharide gums, some used for enhanced oil recovery. Now there would appear to be another potential for such products.

Temperature affected cadmium biosorption in all the bacteria used, but only at a minor level which would seem to suggest that enzyme related processes were not involved in cadmium biosorption. In conjunction with this observation, the biosorption of cadmium by live and dead biomass showed that most cadmium binding did not appear to be predominantly associated with an active process. Both Arthrobacter species and both pseudomonads bound cadmium intracellularly, but this was apparently not predominantly a metabolically linked phenomenon.

With increase in hydrogen ion concentration, cadmium biosorption by the bacterial species employed decreased. This effect has been observed with many other biosorbents (Gadd, 1986; Shumate and Strandberg, 1985) and would appear to be due to displacement of bound metal ions by hydrogen ions and vice versa. Similarly, when other divalent heavy metal cations were added to

solutions containing cadmium ions the biosorbents employed exhibited a lower cadmium binding capacity, indicating that some common sites existed for binding cadmium and the other divalent metal ions. However, the overall amount of metal bound also increased in mixed solutions compared to cadmium solutions of equivalent total metal concentration, suggesting that other metal ions present could occupy sites not available to cadmium ions. Zinc is chemically very similar to cadmium, but zinc binding observed was not always equivalent to cadmium binding. Other ions less chemically similar to cadmium were often accumulated better than cadmium (Pb^{2+} and Cu^{2+} for example). It is apparent that cadmium binding by these biosorbents is not cadmium specific to any major degree.

The effect of cadmium exposure of biomass on cell viability indicated that capsule forming K.aerogenes strains were more resistant than strain 9528. Both Arthrobacters appeared quite resilient and the pseudomonads were most susceptible. That A.viscosus possessed no extracellular capsule, yet withstood cadmium exposure as well as the encapsulated K.aerogenes strains, and better than A.globiformis, supports the proposal that it possesses a cadmium detoxification mechanism, which from electron microscopy and x-ray spectroscopy would appear to be linked with phosphorus.

Recovery of cadmium from the biosorbents A.viscosus and P.cruciviae demonstrated that of the mineral acids hydrochloric acid was the best eluant. Sodium hydrogen carbonate and sodium carbonate presented practical problems if used as desorption agents. The best desorption agent was citric acid. This may be attributed to its ability to displace cadmium ions by hydrogen ions and remove cadmium ions in solution by complexation. As A.viscosus bound cadmium intracellularly, it might have been expected to be difficult to recover cadmium without disruption of the cell, but this did not appear to be the case. Citric acid achieved total elution of cadmium bound to A.viscosus, an important consideration for process application. Elution of cadmium by hydrochloric acid was found to be as rapid as cadmium uptake. When recovery of cadmium from A.viscosus, P.putida and K.aerogenes was achieved using biomass with increasing cadmium loadings, the efficiency of recovery of the metal decreased for all but K.aerogenes. This has been attributed to the lower amounts of metal present on K.aerogenes biomass in these experiments (Section 3.3.3). But it could alternatively be due to cadmium accumulation by A.viscosus and P.putida being predominantly intracellular, whereas K.aerogenes 9128 showed little intracellular uptake of cadmium.

Tsezos (1986) has identified many of the problems associated with use of live biomass for metal recovery. These problems could be addressed and rendered insignificant under the correct operating conditions (Section 1.6) and proper bioreactor. Application of live biomass to cadmium recovery within a fluidised bed has proved impractical (Section 3.4).

Despite the fluidised bed being an attractive bioreactor with respect to the area it occupies and volume of effluent it can treat, retention of live biomass on support material was poor. Such loss of biosorbent from the system, plus the maintenance that would be associated with a continuous operation (culture maintenance) utilizing live biomass implies that the fluidised bed is not the appropriate type of bioreactor for cadmium recovery by live biomass. A further problem associated with use of live biomass in metal recovery is that metal elution is required, and must by necessity be very effective, otherwise waste disposal problems are encountered.

Such arguments do not invalidate use of live biomass for metal recovery under properly controlled conditions. The most cost-effective waste-water decontamination yet achieved is provided by algae in the settling ponds

and meander systems employed by some mining companies (Section 1.1). Furthermore, provided toxic metal can be efficiently chemically eluted, or otherwise cheaply and efficiently recovered from the biosorbent, then the waste could be used as fertilizer. Under a correctly operated process the adsorbent itself can be easily discarded in a biological system.

Dead biomass has been employed within a fluidised bed for waste-water decontamination (Brierly et al, 1986). However, even when treated and compacted, dead biomass is still of low density which presents problems for application within a fluidised bed required to treat large volumes of waste-water.

Although use of live biomass within a fluidised bed was not found suitable for a cadmium recovery process in this investigation, this does not necessarily invalidate use of live biomass as a biosorbent. Different types of contacting system that did not require the use of support particles could negate the problems of biomass attrition .

Apart from being self-regenerating , another potential advantage to the use of live biomass is that a dual treatment system might be devised , where for example , bacteria capable of nitrate reduction or phenol degradation could be used as biosorbent .

At present few studies have been made on the operating efficiency and cost-effectiveness of using live biomass for toxic metal recovery on a pilot scale or large scale. The ultimate aim of characterising biosorbents is to produce a waste-water treatment process for heavy metal recovery that is competitive with currently existing processes . To achieve this capital cost and maintenance costs of such such systems need to be assessed and the systems designed to achieve lower costs than , and be more as or more effecient than currently employed processes otherwise biological metal recovery has little future in treatment of heavy metal laden waste-water .

4.1 Further Work

From the data gathered during this study, several recommendations for further investigation can be made.

1. Sites of biomass cadmium accumulation

The sites of accumulation identified by electron microscopy and x-ray spectography at 1000mg/l initial cadmium might not be so prominent, or might be different, from those occupied at lower cadmium concentrations. This may be particularly pertinent to the bacterial species that apparently possessed some detoxification mechanism. Thus these analyses need to be repeated at other cadmium concentrations, particularly for K.aerogenes, where little cadmium was found within the cell and the location of the cadmium removed from solution was not definitely elucidated. In addition, corroborative analyses need to be carried out, such as cell disruption to separate cell components, and differential centrifugation to isolate samples of the components for separate addition to cadmium to determine which bind most cadmium.

2. Biosorption Kinetics

Rapidity of cadmium biosorption made it impossible to determine accurately the rate order of the biosorption process by analyses in which biosorbent was separated from cadmium solution by centrifugation. To follow the

reaction fully, a cadmium monitoring technique that rapidly determines cadmium levels in solution without being compromised by the presence of biomass is required. Separation of biomass from cadmium solution occupies too great a time period with respect to the speed of cadmium uptake. Addition of biomass to the polarography vessel was attempted on several occasions, but presence of biomass often interfered with polarography and this method of analysis was abandoned. In addition, a time lag occurred between the initiation of a polarographic determination of cadmium and obtaining the result (i.e. chart peak). It is suggested that a cadmium specific ion selective electrode be employed in a rate investigation as this would give a virtually instantaneous response via a digital monitor. Biomass would then be added to a standard solution and the removal of cadmium from solution monitored from the time of addition.

3. Applications of exopolysaccharide as a biosorbent

As previously stated, microbially produced polysaccharide has potential as a biosorbent. Apart from the polysaccharide produced by A.viscosus strain NCIMB 9728 (investigated here), the cadmium biosorption by polysaccharide excreted by A.viscosus strain NCIMB 9729 and that of other bacteria cultivated for this purpose in industry should be investigated. The most

suitable polymer would have the highest cadmium biosorptive capacity, and be insoluble to prevent loss of biosorbent. Effects of dehydration on polysaccharide also needs to be closely investigated as this appears to significantly reduce biosorptive capacity for metal (Section 3.2.9).

4. Bioreactor Efficiency

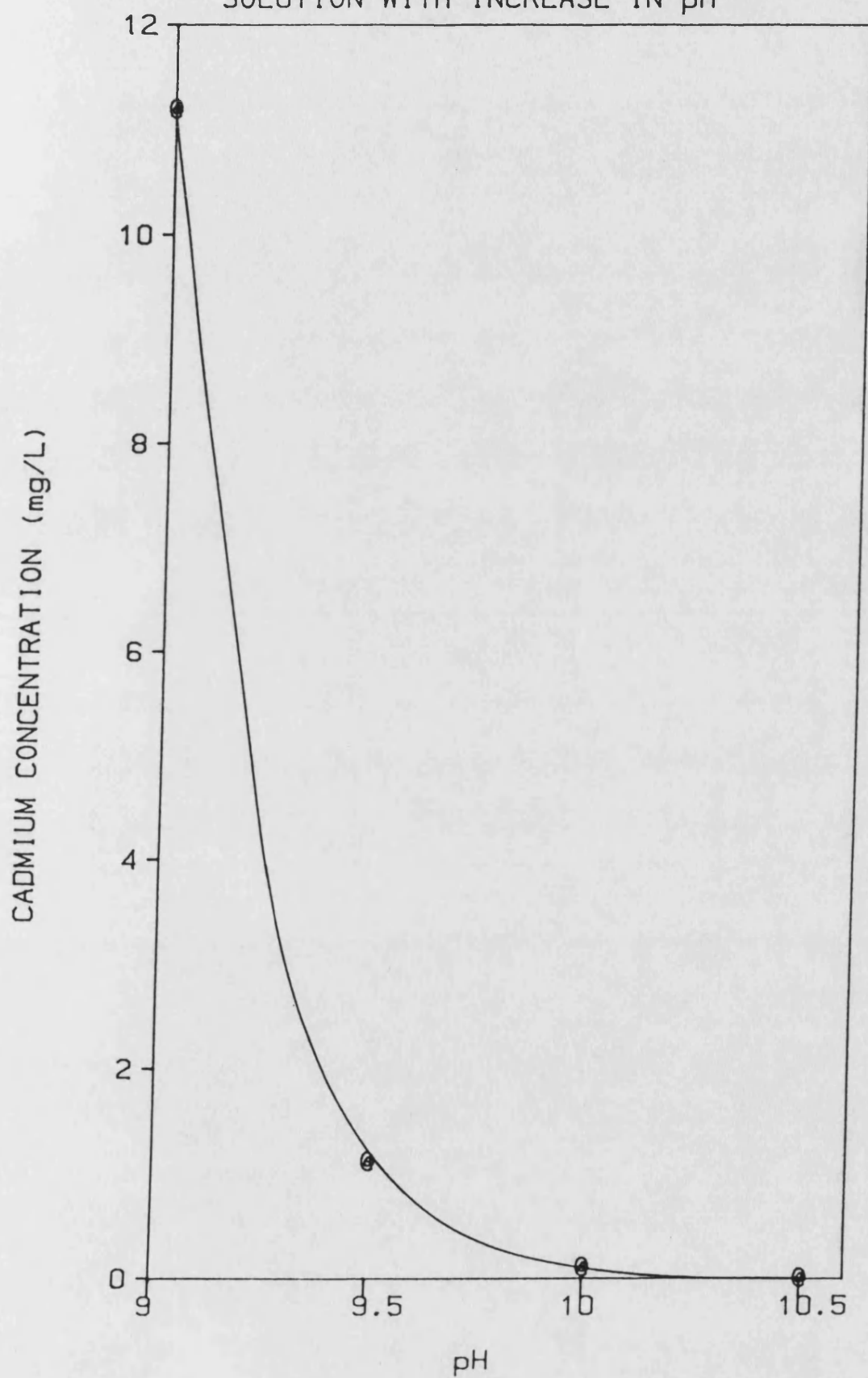
Despite their advantages, fluidised beds employed in conjunction with live biomass are inappropriate due to attrition of biomass. Comparison of the operating efficiency of fermenters, packed beds and large flow-through tanks would provide valuable data to allow elucidation of which system worked best under various operating conditions. Such a study would also need to compare potentially self-regenerating systems using live biomass to systems using dead biomass, and the use of contacting devices in series investigated. High efficiencies of removal ($>99\%$) that would be required in operation of a biological recovery system (Brierly *et al*, 1986) could be achieved using bioreactors in series. Finally, in investigating such systems, the capital and maintenance costs of the systems need to be established to determine which is most cost-effective.

5. Appendix.

Solubility of cadmium beyond pH 9

As solution pH increases above pH 9 the solubility of cadmium rapidly decreases (Figure 48).

FIG.48 DECREASE OF CADMIUM CONCENTRATION IN SOLUTION WITH INCREASE IN pH



6. References.

Allen, D.A.; Austin, B.; Colwell, R.R. (1977) Antimicrob. Agent Chemo. 12:545-547

Anon. (1977) Plating and Surface Finishing : Report on National Bureau Stat. Meet. on Cadmium Plating. pp9-14

Aiking, H.; Kok, K.; Heerikhuizen, H.; Riet, J. (1982) Appl. Environ. Microbiol. 44(4):938-944

Aiking, H.; Stijnman, A.; Garderen, C.; Heerikhuizen, H.; Riet, J. (1984) Appl. Environ. Microbiol. 47(2):374-377

Brierly, C.L. (1982) Sci. Am. 247:44-53

Brierly, C.L.; Brierly, J.A. (1980) In: Biogeochemistry of ancient and modern environments . Trudinger, P.A.; Walter, M.R.; Ralph, B.J. (eds). p661 Springer-Verlag (1980)

Brown, M.J.; Lester, J.N. (1979) Wat. Res. 13(9):817-838
Biotechnology (1983) 1(3):228-237

Brierly, J.A.; Goyak, G.M.; Brierly, C.L. (1986) In Immobilisation of ions by bio-sorption. Eccles, H.; Hunt, S. (eds). p105-120. Publ. Ellis-Horwood.

Babich, H.; Stotzky, G. (1977) Appl. Environ. Microbiol. 33(3): p681-695

Bitton, G.; Freihofer, V. (1978) Microb. Ecol. 4:119-125

Beveridge, T.J.; Murray, R.G.E (1976) Journ. Bacteriol. 127:

p1502-1518

Beveridge, T.J.; Koval, S.F. (1981) Appl. Environ. Microbiol.
42(2):325-335

Beveridge, T.J.; Murray, R.G.E. (1980) J. Bacteriol. 141:
p846-887

Beveridge, T.J. ; Forsberg, C.W. ; Doyle, R.J. (1982)
J. Bacteriol. 150:1438-1448

Bauda, P.; Block, J.C. (1985) Environ. Technol. Lett. 6(10):
p445-454

Corpe, W.A. (1975) Dev. Ind. Microbiol. 16:249-255

Dart, M. (1982) Thames Water Prog. in Research (1981-2) p18

Dugan, P.R.; Pickrum, H.M. (1972) Perdue Univ. Eng. Ext.
Ser. Bull. 141:1019-1038

Doyle, R.J. ; Matthews, T.A. ; Streips, U.N. (1980)
J. Bacteriol. 143:471-480

deRome, L.; Gadd, G.M. (1987) Appl. Microbiol. Biotech.
26:84-90

Dudman, W.F.; Wilkinson, J.F. (1956) Biochem. Journ. 62:p289-

Ehrlich, H.L. (1978) In Microbial Life in Extreme
Environments. Kushner, D.J. (ed): p381-408. Publ. Academic
Press

Ensign, J.C.; Wolfe, R.S. (1964) J. Bacteriol. 87:924-932

- Foster, P.J. (1983) Chem. Eng. 73:p392
- Friis, N.; Myers-Keith, P. (1986) Biotech. Bioeng. 28:21-28
- Friedman, B.A.; Dugan, P.R. (1968) Dev. Ind. Microbiol. 9:
p381-388
- Gale, N.L.; Wixson, B.G. (1980) Dev. Ind. Microbiol. 20:
p259-273
- Gadd, G.M.; Griffiths, A.J. (1978) Micro. Ecol. 4:303-317
- Gadd, G.M. (1986) In : Immobilisation of ions by
bio-sorption. p149-159. Eccles, H.; Hunt, S. (eds).
- Gadd, G.M.; Mowll, J.L. (1983) F.E.M.S. Microbiol. Lett.
16:45-48
- Gasdorf, H.J.; Benedict, R.G.; Cadmus, M.C.; Anderson, R.F.;
- Jackson, R.W. (1965) J. Bacteriol. 90:147-150
- Giles, C.M.; McEwan, T.H.; Nakhwa, S.; Smith, D. (1960)
Journ. Chem. Soc. 3973-3993.
- Hardy, K. (1983) In : Bacterial Plasmids. p62-3, 71-2.
Publ. Nelson.
- Horikoshi, T.; Nakajuma, A.; Sakaguchi, T. (1981) Eur. Journ.
Appl. Microbiol. Biotech. 12:90-96

- Horitsu, H.; Nishida, H.; Kato, H.; Tomoyeda, M. (1978) Agric. Biol. Chem. 42(11):2037-43
- Horitsu, H.; Kato, H.; Tomoyeda, M. (1979) Journ. Ferment. Technol. 57(4):273-79
- Higham, D.P.; Sadler, P.J.; Scawen, M. (1985) Journ. Gen. Microbiol. 131:2539-2544
- Iverson, W.P.; Brinckman, F.E. (1978) In : Water Pollution Microbiology 2p201-232. Mitchell, R. (ed) Publ. Wiley & Sons
- Jilek, R.; Fуска, J.; Nemec, P. (1978) Biologia 33(3):201-7
- Kelly, D.P. ; Norris, P.R. ; Brierly, C.L. (1979) In : Microbial Technology: Current State, Future Prospects Bull, A.T.; Ellwood, C.; Ratleds, C. (eds). 29th Symp. Soc. Gen. Microbiol. (1979).
- Khazaeli, M.B.; Mitra, R.S. (1981) Appl. Environ. Microbiol 41(1):46-50
- Kiff, R.J.; Little, D.R. (1986) In : Immobilisation of ions by bio-sorption. p71- Eccles, H.; Hunt, S. (eds).
- Keddie, R.M.; Jones, D. (1983) In : The Prokaryotes. 2 Chapt. 142:1842-1851
- Lauwerys, R. (1979) In : The Chemistry, Biochemistry, and Biology of Cadmium . Webb, M. (ed) Ch. 11:433-452

Lester, J.N. ; Harrison, R.M ; Perry, R. (1979) Sci. Tot.
Environ. 12:13-23

Manchee, R. (1979) Trends. Biochem. Sci. 4(4):77-80

Maxwell, W.A.; Metzler, R.; Spoerl, E. (1971) J. Bacteriol.
105:1205-1206

Mangi, J.I.; Schumacher, G.J. (1979) Am. Midl. Nature
102:134-139

Muzzarelli, R.A.A.; Bregani, F.; Sigon, F. (1986) In :
Immobilisation of ions by bio-sorption. p183-201
Eccles, H.; Hunt, S. (eds)

McReady, R.G.L.; Lakshmanan, V.I. (1986) In :
Immobilisation of ions by bio-sorption. p219-227
Eccles, H.; Hunt, S. (eds)

Mance, G. (1984) Chem. and Ind. p509-513

Milne, R. (1987) New Scientist 116:(1587)p53-

Mitra, R.S.; Gray, R.H.; Chin, B.; Bernstein, I.A. (1975)
J. Bacteriol. 121:1180-1188

Macaskie, L.E.; Dean, A.C.R. (1984) Environ. Technol.
Lett. 5:177-186

Miller, C.L.; Landa, E.R.; Updegraff, D.M. (1987) Microb.
Ecol. 14:141-155

Nakahara, H.; Tomoaki, I.; Sarai, Y.; Kondo, I.; Mitsuhashi, S.
(1977) Nature 266:165-167

Nakajuma, A.; Horikoshi, T.; Sakaguchi, T. (1982) J. Microb.
Biotech. 16:88-91

Norberg, A.B.; Persson, H. (1984) Biotech. Bioeng. 26:
p239-246

Peterson, P.J.; Alloway, B.J. (1979) In : The Chemistry
Biochemistry and Biology of Cadmium. p45-46. Webb, M. (ed)

Pahren, H.R.; Lucas, J.B.; Ryan, J.A.; Dotson, G.K. (1979)
Journ. Wat. Poll. Contr. Fed. 51:2588-2601

Pickett, W.; Dean, A.C.R. (1976) Microbios 15:79-91

Ruchloft, C.C.; (1949) Sew. Treat. Works Journ. 21:877-883

Rahut, A. (1978) Methods and Costs of Preventing Cadmium
Emissions. Zinc Development Assc. / Cadmium Assc.
34 Berkeley Square, London W1X 6AJ.

Rudd, T.; Sterritt, R.M.; Lester, J.N. (1984) Wat. Res. 18(3)
p379-384

Ross, I.S.; Townsley, C.C. (1986) In : Immobilisation
of ions by bio-sorption. p49 .Eccles, H.; Hunt, S. (eds)

Ryan, T.H. (1984) Lab. Equip. Digest (April, 1984).

Samarawickrama, G.P. (1979) In : The Chemistry

Biochemistry and Biology of Cadmium. Ch.9:341-356

Webb, M. (ed).

Stanier, R.Y.; Adelberg, E.A.; Ingraham, J.L. (1979) In :
General Microbiology (4th Edn) Ch.2:28-29.

Publ. Prentice-Hall.

Sterritt, M.; Lester, J.N. (1980) Sci. Tot. Environ. 14:
5-17.

Silver, S.; Misra, T.K. (1983) In : Genetic Control of
Environmental Pollutants. p23-46. Omen, D.; Holleander, S.
(eds). Publ. Plenum Press

Shumate, S.E.; Strandberg, G.W. (1985) In : Comprehensive
Biotechnology 4:Ch.13 p235-. Robinson, C.W.; Howell, J.A.
(eds).

Strandberg, G.W.; Shumate, S.E.; Parrott, J.R. (1981) Appl.
Environ. Microbiol. 41:237-

Sterritt, M.; Lester, J.N. (1986) In : Immobilisation of
ions by bio-sorption. p135-149. Eccles, H.; Hunt, S. (eds).

Sakaguchi, T.; Horikoshi, T.; Nakajuma, A. (1978) Journ.
Ferment. Technol. 56:561-565

Slonecker, J.H.; Orentas, D.G.; Knutson, C.A.; Watson, P.R.,
Jeanes, A. (1968) Can. J. Chem. 46(21):3353-3361

Scott, J.A.; Palmer, S.J. (1988) *Biotech. Lett.* 10: 21-24

Scott, J.A.; Palmer, S.J.; Ingham, J. (1986) *In* :
Immobilisation of ions by bio-sorption. p81-. Eccles, H.;
Hunt, S. (eds).

Scott, J.A.; Palmer, S.J.; Ingham, J. (1986) *Effl. Treat. Dis.*
(I. Chem. E. Symp. 96) p211-220

Thompson, K.C.; Prior, B.F. (1984) *Effl. Wat. Treat. J.*
24: 196-200

Tsezos, M.; Volesky, B. (1981) *Biotech. Bioeng.* 23: 583-

Tuovinen, O.H.; Kelly, D.P. (1974a) *Arch. Microbiol.* 95
: 153-164

Tuovinen, O.H.; Kelly, D.P. (1974b) *Arch. Microbiol.* 95
: 165-180

Tsezos, M.; Volesky, B. (1982a) *Biotech. Bioeng.* 24:
p835-840

Tsezos, M.; Volesky, B. (1982b) *Biotech. Bioeng.* 24:
p955-967

Tuovinen, O.H.; Dispirito, A.A. (1984) *In* : *Current*
Perspectives in Microbial Ecology. p608-614

Tsezos, M.; Keller, D.M. (1983) Biotech. Bioeng. 25:
p201-217.

Tsezos, M. (1984) Biotech. Bioeng. 26:973-981

Updegraff, D.M.; Douros, J.D. (1971) Dev. Ind. Microbiol.
13:76-90

Webb, M. (1979) In: The Chemistry, Biochemistry and
Biology of Cadmium. Ch. 6, 8 & 10. Webb, M. (ed). Publ.
Elsevier/North-Holland.

Wilkinson, J.F. (1957) Bact. Rev. 22:46-72

Yakuba, N.A.; Dudeney, A.W.L. (1986) In :
Immobilisation of ions by bio-sorption. p201-219
Eccles, H.; Hunt, S. (eds)

Zajic, J.E.; Chiu, Y.S. (1971) Dev. Ind. Microbiol.
13:91-100
